JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXX WASHINGTON, D. C., FEBRUARY 15, 1925 No. 4

# ROOT ROT OF PEAS IN THE UNITED STATES CAUSED BY APHANOMYCES EUTEICHES (N. SP.) [1, 2]

By FRED REUEL JONES, *Pathologist*, and CHARLES DRECHSLER, *Associate Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

During the past five years, in which the senior writer has endeavored to determine the causes which produce the root rot of peas known widely in the United States, several fungous diseases have been distinguished which severally or together appear to be responsible for all of the important injury which has been found incident to the underground portion of this plant. One of the less important of these diseases caused by a species of Fusarium has been described in a previous paper (*8*).[3] The most important of these diseases, more important, in fact, than the others combined, is the subject of the present paper. The account of the morphology and taxonomy of the fungus causing the disease and the drawings are contributed by the junior author. Several fungous diseases of pea roots of much local importance have also been studied. Among these are two caused by species of Pythium, one of which in some seasons is almost always associated with Aphanomyces, an association which misled the senior writer in a previous note (*6*) to ascribe to the Pythium alone the injury due to both organisms. The diseases caused by species of Pythium remain to be described in a following paper.

## THE DISEASE

### DESCRIPTION

The injury caused by a fungous parasite which invades only the subterranean portion of the plant must, of course, be sought in its early stages in the roots themselves. In later stages the top of the plant may be modified in response to the root injury in a way which may be specifically characteristic. In the case of this disease the injury eventually presented by the top of the plant is not characteristic, the form which it takes depending largely upon the stage of development at which the roots became thoroughly invaded and to a lesser extent upon the degree of resistance of the variety of peas. If the plant becomes invaded in the basal stem below ground as well as in the roots before the plant has developed more than three or four nodes, sudden wilting may result. Usually under field conditions invasion takes place later than this, and the result is a general retardation of growth, the death of the lower leaves progressively upward, and finally, when the plant is in full bloom, it may shrivel up completely. More frequently, however, the plant persists in a weakened condition until it has brought its poorly filled pods to maturity.

If extensive infestation of the roots is delayed until the blossoming period, the plants may mature under favorable conditions without any conspicuous indication of injury. These several symptoms have no common factor which distinguishes plants infected with Aphanomyces from those attacked by several other fungi. However, there is one test which can be applied that will often give a decisive indication of this disease. If some of the infested plants are pulled, the stems of those which are thoroughly invaded will fail to break at the seed, as is the usual rule with healthy plants, but the vascular core of the taproot will pull out as a long string

[1] Received for publication May 19, 1924—issued April, 1925.
[2] Results of work done in cooperation with the Agricultural Experiment Station of the University of Wisconsin.
[3] Reference is made by number (italic) to "Literature cited," p. 325.

with a few of the larger lateral branches. No other type of root rot has been found which permits the pulling out of these vascular strings. This test does not apply in the earlier stages of disease and sometimes fails to give results when the soil is exceedingly hard and dry. Examination of the roots themselves will reveal the extent of injury by the soft decay of the cortex. In early stages there is a pale yellow discoloration of the decayed tissue not readily distinguishable from the discoloration associated with the invasion of the mycorrhizal fungus described in a previous paper (*9*).

The soft decay and shrinking of the cortex of the roots and of the portion of the stem below ground usually distinguishes this disease from the turgid roots with the mycorrhizal fungus. After a time the dead cortical tissue usually becomes blackened (pl. 1) so that it can not readily be distinguished by this character from other forms of root decay. If the visible symptoms already described fail to distinguish the disease with certainty, it is almost always possible at any stage in the development of the disease to distinguish it by an examination of the roots with a microscope. The characteristic spores of the fungus, described later, are always formed more or less abundantly in some of the dead cortex of root (pl. 2) or more rarely in that of the stem, and serve to determine the presence of this parasite beyond possible doubt.

### HISTORY OF THE DISEASE

Since this disease has not been distinguished previously under any name, it has no unmistakable written history. There are a few references to pea-root troubles, ascribed to other fungi, but on such insufficient evidence that it is not impossible that they may be pertinent here. The first of these is by Wittmack [4] (*19*), who found in decaying roots of peas sent to him by Sadebeck from Hamburg, Germany, oospores apparently belonging to a new Pythium, which he called *P. sadebeckianum*.

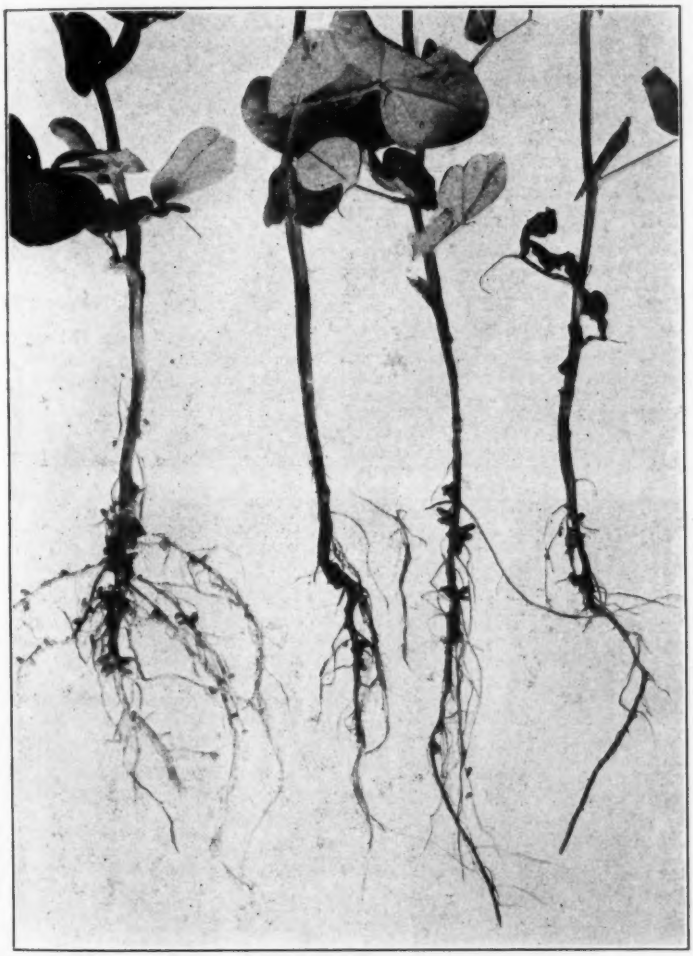
In the United States the disease has undoubtedly been present for a long time and has compelled the abandonment of intensive culture of peas for canning purposes in certain restricted areas. Recently Clinton (*2*) has described a root rot of peas in Connecticut evidenced by the presence of oospores which he believed to be those of *Phytophthora cactorum*. The senior writer has been unable to produce any extensive decay in pea roots growing in soil with a culture of this species, and in no case were oospores formed in the few small lesions produced. Although infection of root ends with a pure culture of a species of Pythium has been obtained by the writer with the formation of oospores in the decaying tissue, and though spores apparently of this species have been found occasionally in field material, nevertheless the measurements and drawings given by Clinton appear for the most part to agree more closely with the spores of the Aphanomyces species, to which reference was made in a brief abstract (*7*) and which is fully described in this paper, than with those of any other fungus that has been encountered. There are other references to rootrot of peas in the United States which undoubtedly indicate this disease, but which do not contain a sufficiently adequate description to make exact determination possible.

## THE FUNGUS

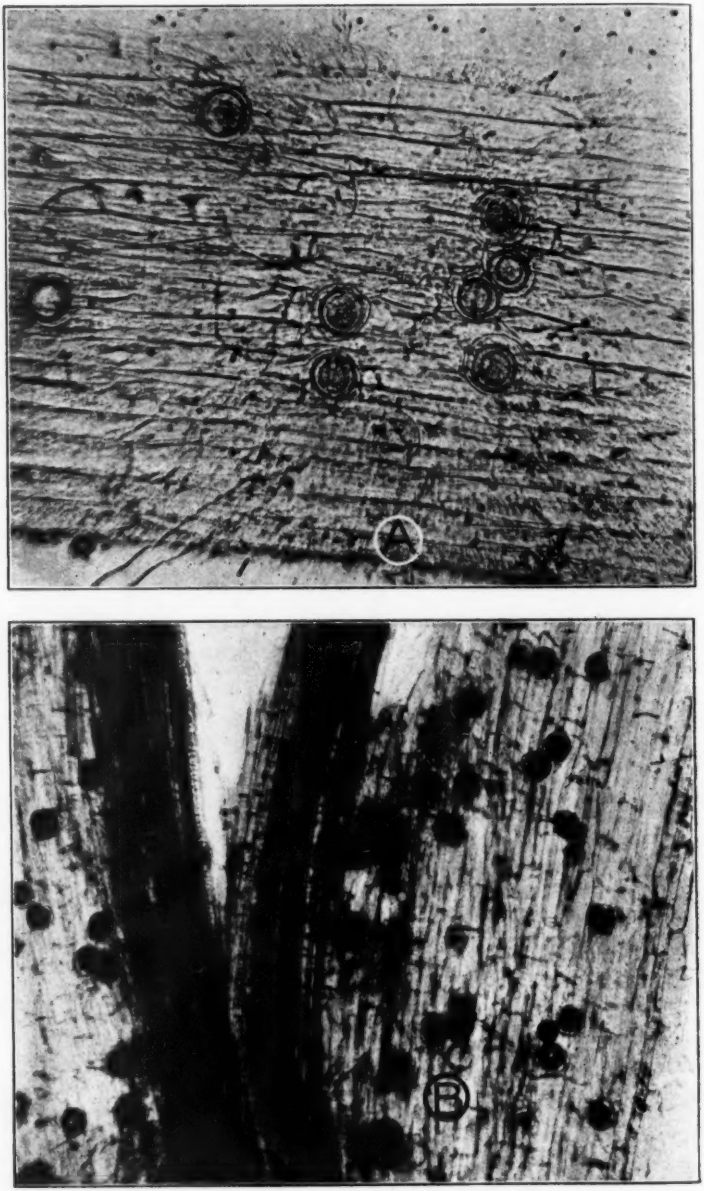
### HOST PLANTS

Although it seems unlikely that this fungus is parasitic only on peas, a search for other host plants has thus far been in vain. The roots of many species of plants growing among diseased peas have been examined for the presence of the characteristic oospores and decayed cortex which might have been produced by this fungus. In order to test thoroughly the possible relation of this fungus to the species of Aphanomyces on sugar beets, described by Peters (*13*, *14*), seedlings of this plant and of cress (*Lepidium sativum*) have been repeatedly inoculated without any infection resulting. The conclusion that these fungi are distinct, at least in their physiological capacities, is further borne out by the fact that examination made of fields of beets grown on pea-sick soil, made both by the senior writer and R. E. Vaughan, has discovered no trace of the European disease. On the other hand, although it appears generally true that beets grow well on old pea fields and vice versa, experience is reported otherwise in the Salt Lake Valley, Utah. It may be of interest to note in this connection that in the course of making isolation from diseased peas from this valley with the

[4] WITTMACK, L. UEBER EINE DURCH PYTHIUM VERANLASSTE KRANKHEIT DER ERBSENWURZELN. Paper presented at 64. Versamml. zu Halle, Gesell. Deut. Naturf. u. Aerzte, Sep. 1891. Not published. Title in Verhandlungen 2: 108. 1892.]



Root rot of peas, variety Early Market, caused by *Aphanomyces euteiches* occurring in a field at Hanover, Md., May 21, 1920. The plant at the left with white clean stem and turgid roots is not infested. The three remaining plants show increasing degrees of disease progressively toward the right. The plant at the extreme right shows the first symptoms of disease above ground in the shriveled lower leaf




Oospores of *Aphanomyces euteiches* in cortex of the roots of peas inoculated with a pure culture of the fungus

A.—Oospores in a razor section. × about 300
B.—Oospores as they appear in a decaying rootlet crushed under a cover glass. × about 150

writer, John W. Carlson, of the Utah Experiment Station, obtained among other fungi a culture of *Pythium* (*Rheosporangium*) *aphanadermatum* (Edson) Fitzpatrick, one of the well-known sugar-beet parasites. This is one of the species of Pythium which the writer has found capable of producing root rot of peas, and if it is widely distributed in this valley it may be the common parasite of these plants which renders pea growing on old beet fields unprofitable.

## GEOGRAPHIC DISTRIBUTION AND ECONOMIC IMPORTANCE

In the United States the disease has been found in practically every pea-growing district that has been searched with care. In the Eastern and Central States it occurs frequently, and often very destructively. It has been found in Utah, Idaho, and Montana, where it appears to be unimportant at present except under special conditions described later. In the Pacific Coast States it has been found but once, in diseased pea plants sent from Santa Clara, Calif. It appears that, although the disease is very widely distributed, it requires special soil and climatic conditions generally present only in the Eastern and Central States in order to become important when peas are grown intensively. It is probably this disease more than any other factor which has compelled the growing of peas in a comparatively long rotation, and has thus limited the culture of this food crop. Certain it is that were it not for the accumulation of this disease and others with intensive pea culture, the cost of producing canned green peas and probably of dried peas would be greatly reduced. In the absence of any accurate survey of fields in the region where this disease occurs, it is impossible to state approximately the number of acres that are damaged or destroyed each year; in some of the older districts in unfavorable years as much as 25 per cent of the acreage seems to be infested. From the reports of growers and county agents, combined with a limited personal survey, it appears that several thousand acres of peas are rendered unprofitable or destroyed in the United States each year.

## ISOLATION OF THE FUNGUS

The fact that this important parasite of peas has remained undescribed so long is undoubtedly due to the difficulties encountered in isolating it in pure culture. One cause of failure is due to the brief period of time during which the fungus flourishes in an active vegetative stage at any point in the host tissue. When the fungus first begins to invade tissue, it can hardly be induced to grow out on the culture medium in preference to the living cells. Only a few days are required at ordinary temperatures to exhaust and destroy the host cells, whereupon the mycelium begins to transfer its contents into the large oospores, which are formed abundantly. After this process is well under way it is again almost impossible to secure growth on culture media. Thus it is necessary to obtain diseased plants in which the fungus is growing vigorously just prior to extensive oospore formation if success in isolation is to be attained.

Another cause of failure is due to the large number of vigorous saprophytes which follow closely the invading Aphanomyces. Besides abundant bacteria, in some seasons there is almost always present a species of Pythium so much more vigorous in growth upon culture media that it usually submerges the Aphanomyces. As yet no method of surface disinfection tried has given material aid in destroying the saprophytes present. The parasite is extremely sensitive to bichloride of mercury, and its use almost invariably brings failure.

In order to secure cultures of the fungus from localities remote from facilities for making isolations or from plants which are so far decayed that direct isolation is impossible, the senior writer has been accustomed to pack diseased roots with soil from the field in tin cans kept tightly sealed awaiting a convenient time in which to combine this material with steam-sterilized soil. In this soil mixture peas are then grown properly protected from outside infection until plants are obtained which have developed the stage of the disease suitable for the isolation of the parasite. In this way cultures have been obtained for comparison from nearly all of the regions where the disease has been found. Fragments of tissue are selected in which the mycelium of the fungus is seen under the microscope to be filled with granular contents, are thoroughly washed in sterile water, and are placed on plates of 2 or 3 per cent clear agar, or preferably prune agar, as recommended by Hartley (*4*) for the isolation of Pythium. The parasite always grows sparsely, sending out long, straight filaments with comparatively short lateral branches through or over the agar. It will almost always outgrow the bacteria which soon develop abund-

antly. Fragments of agar containing ends of these strands are cut from the plate and transferred to new plates until no bacterial growth accompanies the fungus, when it is transferred to corn-meal agar for a stock culture. It often happens that strands of Pythium grow in a manner resembling Aphanomyces so closely that they can not be distinguished until they are transferred to new plates of prune agar, where they soon develop a white matted growth very different in character from the sparse arachnoid growth of Aphanomyces. Oftentimes many fragments of roots and stems must be selected and plated before a culture of the desired fungus is obtained.

## MORPHOLOGY OF PARASITE

### MYCELIUM

In longitudinal sections of diseased pea stems the vegetative stage of the parasite is revealed as a hyaline nonseptate mycelium, composed of hyphae, varying considerably in diameter among themselves, but individually not subject to abrupt fluctuations in respect to this dimension. (Pl. 3, A.) Branching is exhibited in moderate, usually not in great abundance, the branches generally being produced at angles approaching a right angle. Not infrequently branches show little linear growth, then remaining as short diverticulate spurs on the axial filaments. The fungus is largely intracellular, the hyphae being oriented longitudinally within the cells, their development between the cells being relatively meager and apparently more or less accidental. Appearances often suggest that the fungus passes through the cell walls of the host perhaps with less ease than, for example, some parasitic species of Pythium. Whereas in cortical tissue invaded by the common damping-off fungus, the hyphae pass promiscuously from cell to cell, without much evidence of the membranes providing any obstacle, in tissue invaded by the root rot parasite cells crowded with mycelium may lie adjacent to others entirely free of the fungus. It should be mentioned, however, that the cortical tissue invaded by the root rot parasite is of a distinctly less succulent character than the cortical tissue of seedlings of various hosts subject to damping-off, and that strains of Pythium effective in producing the latter type of injury exhibit little or no aggressiveness toward pea plants readily attacked by the species of Aphanomyces under consideration. A somewhat similar mycelial distribution was reported by Weatherwax (*17*) in filaments of *Spirogyra dubia* Kg. invaded by *Aphanomyces phycophilus* DeBary.

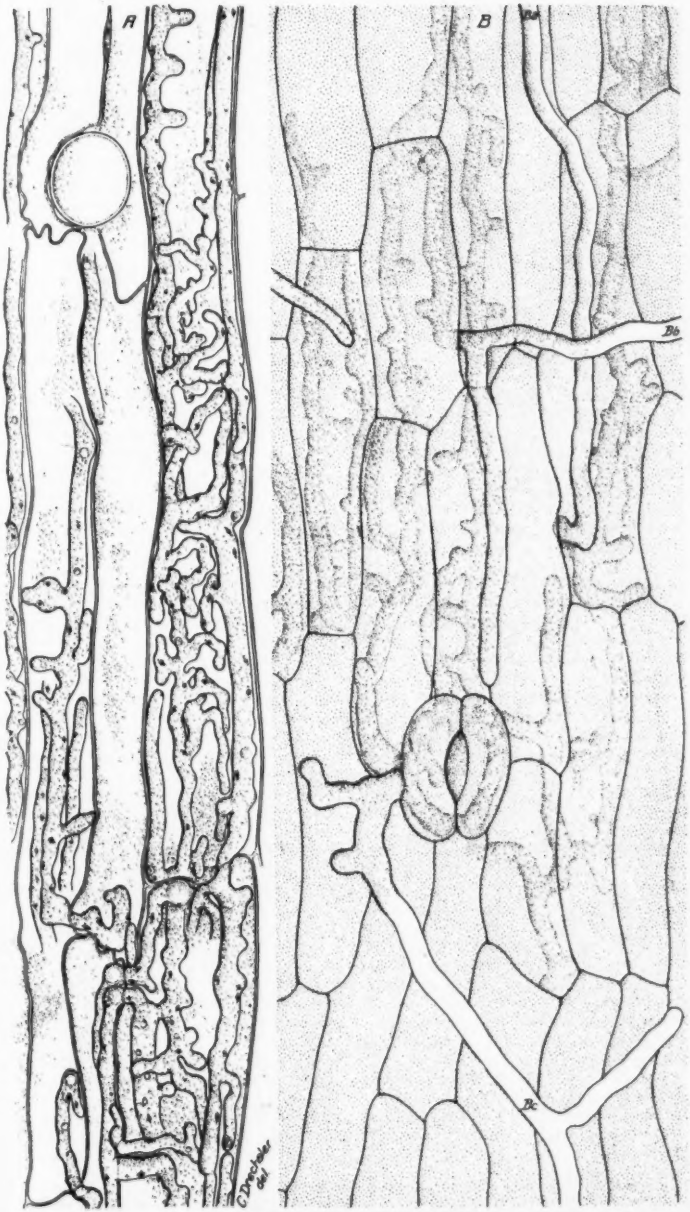
## SEXUAL REPRODUCTION

The purely vegetative condition represents a rather brief phase in the development of the fungus, mycelial growth coming to a pause as the cortical tissue begins to collapse. Oogonia and antheridia now make their appearance. Owing to the crowding of the hyphae within the host cells, the relation between the branches bearing these organs can not usually be distinguished. There is no reason to believe, however, that the sexual apparatus in diseased plants shows any significant departure from these structures as they develop on suitable artificial substrata where they can be accurately studied. The oogonia before fertilization are thin-walled, subglobose bodies with densely granular, vacuolate contents. After fertilization the oogonial wall becomes conspicuously thickened, the thickening being subject to peculiar irregularities, with the result that the inner contour is represented by a more or less sinuous line, giving the whole structure a peculiar internally scalloped appearance. (Pl. 4, A to H.) As in other species of Aphanomyces, the oogonial cavity is very largely but not completely occupied by the single oospore, a subspherical structure with a thick colorless wall, the thickness of the latter not given to great variations either with respect to different individuals or with respect to different portions of the same individual. The contents of the normal mature oospore consists of a

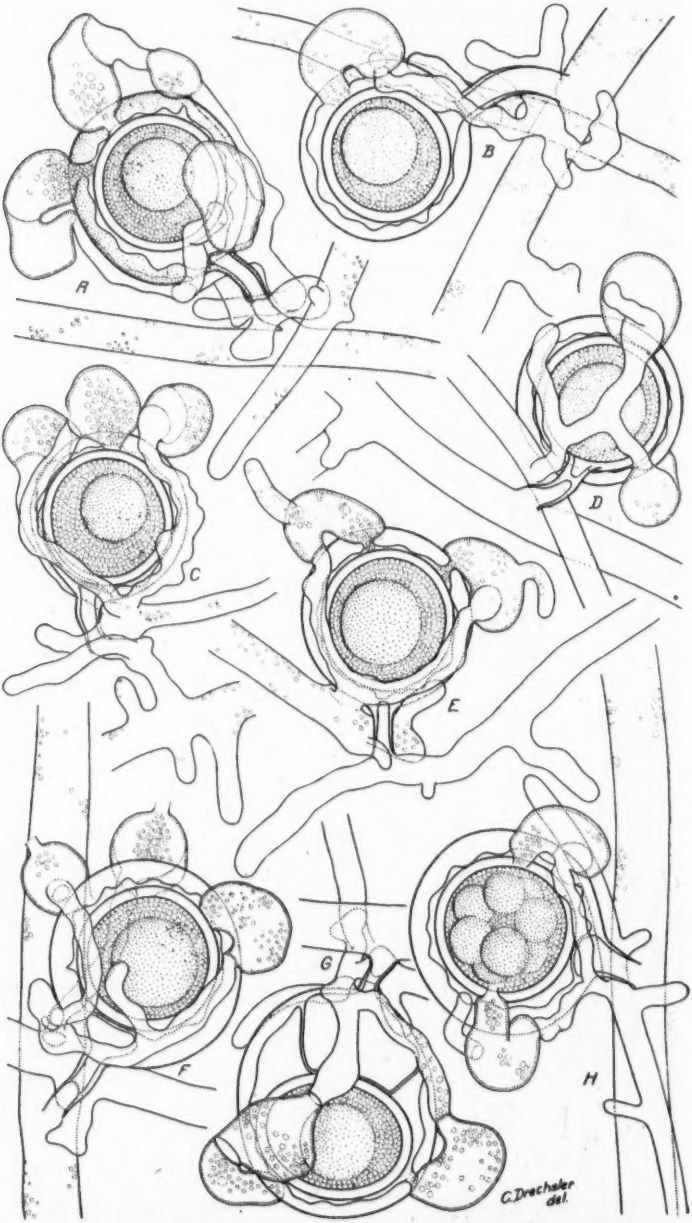
### EXPLANATORY LEGEND FOR PLATE 3

A.—Portion of longitudinal section of basal part of pea stem in an early stage of infection, showing the development of the fungus in certain cortical cells and extension into adjacent cells. The ellipsoidal body near top of figure represents a developing oogonium. From material of a plant grown in soil inoculated with a pure culture of the parasite, killed in Flemming's weaker solution, embedded in paraffin, cut on the microtome, and stained with Flemming's triple combination. ×470

B.—Epidermis of hypocotyl of pea seedling derived from a surface-sterilized seed planted on sterile water agar in a test tube, the resulting culture inoculated 5 days after planting with a pure culture of *Aphanomyces euteiches*. Showing penetration by 3 extramatrical hyphae into tissue of plant—one, *Ba*, entering close to or at the juncture of adjacent epidermal cells; another, *Bb*, entering between two epidermal cells; and a third, *Bc*, entering between a guard cell and an epidermal cell. ×470




(For explanatory legend see p. 298)




Various sexual apparatus of Aphanomyces euteiches from 20 day old hard corn-meal agar cultures, illustrating close approximation of antheridial and oogonial stalks; branching of antheridial stalk; size, shape, and interrelation of oogonia and antheridia; character of cross walls setting of sex organs; and occurrence of diverticulate branches. Drawn with the aid of a camera lucida at a magnification of 1,580 diameters, and reduced in reproduction to a uniform magnification of 470 diameters

large, spherical, somewhat eccentric, vacuole-like body of apparently homogeneous structure, surrounded by a matrix containing numerous small uniform granules in regular concentric arrangement. The literature on the genus Aphanomyces like that on the Saprolegniaceae in general, shows some difference of opinion with reference to the nature of these internal elements, the large central body sometimes being referred to as an oil globule (*1*, *3*, p. 160); while evidently at other times (*3*, p. 10) it is regarded as being of a protoplasmic nature and the granule-like structures as consisting of oily matter. Since the small peripheral granules stain well with Sudan III in the species under consideration, it would seem the latter view is probably more nearly correct, although the apparent homogeneity of the material constituting the central globule can not be regarded as characteristic generally of protoplasm.

The relationships of the sexual apparatus can be studied to excellent advantage when the fungus is grown on hard corn-meal agar. By cutting off a thin surface layer of the substratum, a thin preparation is obtained containing the thallus at nearly a uniform optical level without any serious disruption of the structures concerned. Typically, and at least in the large majority of cases, the male and female organs arise from hyphae which show no close organic connection. The oogonium is apparently always terminal, being generally borne on a rather short stalk arising as a lateral branch from a hypha usually of more than ordinary diameter. The antheridial branch generally arises from a less stout filament, which in many instances will be found to cross the larger filament close to the points from which the sexual branches have their origin. (Pl. 4, A to H.) Usually the antheridial stalk becomes intimately involved with the oogonial stalk after making a partial turn about the latter, although the condition described by Von Minden (*11*) for his *Aphanomyces helicoides*, and figured by Kasanowsky (*10*) in his account of *A. laevis*, in which the antheridial stalk makes several distinct turns about the oogonial stalk, has never been found realized. A number of short diverticulate branches often are borne on the hypha from which the antheridial branch originates (pl. 4, A, B, E, H) and occasionally on the hypha to which the oogonial stalk is attached. (Pl. 4, C, E.) As far as can be determined, they serve no special purpose, but add to the characteristic involved appearance of the apparatus, thereby further enhancing the optical difficulty in resolving it with certainty.

The antheridial branch may be simple or branched, the branching frequently occurring near the base of the oogonium or a short distance from the base. Usually it is limited to a single bifurcation (pl. 4, B, H), but instances in which one (pl. 4, A) or both (pl. 4, F) of the resulting elements branched again have been found, one of the ultimate elements then sometimes failing to develop any male organ. (Pl. 4, D, F, G.) The antheridium is always an expanded structure set off from the stalk by a septum, which is frequently curved with the convexity protruding into the interior. Often the antheridium appears sharply arched in the manner of a measuring worm, the fertilization tube in such instances usually being produced in the region where the distal lobe is in contact with the oogonium. (Pl. 4, B, H.) Not rarely a hyphal diverticulum is present as a dorsal appendage (pl. 4, E, F) quite similar in appearance to analogous protuberances that can be observed occurring singly on the male organs of some species of Pythium and, as in the latter forms, apparently not serving any evident purpose.

In some instances when the antheridium attains unusually large proportions a transverse septum may be inserted, generally at a constriction. (Pl. 4, A, G.) The resulting structure may evidently be regarded either as two male organs developed in series on the same stalk or as a compound antheridium. Sometimes both of the male elements thus delimited have been observed communicating with the interior of the mature oogonium by independent tubes or apertures in the oogonial wall. While the origin of the antheridial branch near the base of the oogonial branch and a sort of contact relation of the two are of common occurrence and characteristic of the fungus, male stalks altogether unrelated to the oogonial stalk in place of origin also occur. As the number of antheridia to an individual oogonium varies from one to four, or even five, considerable variety in origin may be expressed in a single sexual apparatus.

The oogonia and oospores developed in culture are quite similar to those found in diseased host tissue, but a few details not easily observed in the latter substratum may here be studied to advantage. Thus the peculiar thickening of the oogonial wall will be seen produced into the distal portion of the supporting stalk, diminishing rather markedly, so that the latter is repre-

sented in its proximal portion in an entirely unmodified form. An interesting variability is exhibited by the partition delimiting the oogonial cavity at the base. Usually it occurs as a rather inconspicuous cross wall inserted in the distal portion of the stalk, providing no marked interruption in the roughly subspherical interior surface of the oogonial envelope. (Pl. 4, A, F, H.) It sometimes projects into the interior as a convex columella-like structure, and in some cases such development may be so pronounced that the available space is greatly reduced, constraining the oospore to adopt a distinctly ellipsoidal shape, with the major axis transverse to the axis of the oogonium. (Pl. 4, G.) Such modification may perhaps best be regarded as a fortuitous morphological peculiarity rather than as an abnormality, being present in material derived from the diseased host as well as in culture and evidently not adversely affecting the vitality of the oospore.

The processes of fertilization are not as amenable to direct observation as might be desired, for although an abundance of antheridia and oogonia develop readily in liquid culture, and consequently can be obtained in Van Tieghem preparations, their contents appear to degenerate at an early stage, or an oospore will be produced of a patently abnormal character, exhibiting a promiscuous granular or irregularly vacuolate internal structure. Examination of preparations of corn-meal-agar cultures abounding in normal sexual conditions indicate that when several antheridia are present, as is usually the case, all or several of them may develop fertilization tubes. For example, out of three or four antheridia attached to an individual female cell, two or three may usually be found to communicate with the interior of the oogonium by openings through the thick oogonial wall. While not all of the antheridia provided with such communications appear devoid of contents, it is certainly not unusual to find two male organs from which the protoplasm has disappeared completely or almost completely. (Pl. 4, A, F, H.) It is readily apparent that such a condition might be brought about by degeneration quite as well as by evacuation of contents into the oogonium. In a cytological study of a congeneric species, Kasanowsky (*10*) found that after fertilization was effected by one antheridium the nucleus of a second antheridium was intercepted in its passage through the fertilization tube. No statement is made by this author whether or not any transfer of cytoplasmic material may take place from a second antheridium previous to the entrance of the nucleus into the tube and its interception. The empty condition of plural antheridia, frequently encountered, points to such possibility. It is even not inconceivable that where nuclear degeneration is as easily effected as in the sexual apparatus of the coenocytic type, to which Aphanomyces seems to belong, the entrance of a supernumerary nucleus may not bring about as impossible a cytological situation as sometimes has been assumed.

### GERMINATION OF OOSPORES

The germination of oospores of Aphanomyces appears to have been recorded for only two species, DeBary (*1*) having observed the process in material of *Aphanomyces stellatus* De Bary that had been kept in water for three months, and Kasanowsky (*10*) in material of a form he designated as *A. laevis*, DeBary, after this had passed through a resting period of over seven months. In both cases a germ tube was produced which perforated the oogonial wall and developed into a mycelium. A difference in the accounts of these authors may be noted in the promptness with which branching of the germ tube occurred, that of *A. stellatus* giving rise to a large number of hyphae very soon after emerging, if not immediately, while in Kasanowsky's fungus ramification was delayed until the tube had attained a length of 300 $\mu$.

Unlike oospores of the two species mentioned, those of the parasite attacking peas require no extended resting period. When material from 15-day-old corn-meal-agar cultures was transferred to hanging drops in Van Tieghem cells, a considerable proportion of the oospores germinated, the method of germination, whether by mycelial development as observed by DeBary and by Kasanowsky, or by the production of zoospores which has not hitherto been recorded for any member of the genus Aphanomyces, seemingly being dependent to a great extent on the amount of nutrient material incorporated in the preparation. Thus Van Tieghem cultures prepared from material without any preliminary washing or removal of particles of substratum quite invariably exhibited direct germination into a mycelium. When the material was first allowed to soak, and the bits of solid food material removed as completely as possible, indirect germination by means of zoospores predominated. The initial changes which take place during the first 24 hours appear to be the same, regardless of eventual developments. The

thick oospore wall disappears as such, being reduced to a delicate membrane, which yields to the enlargement of the protoplast and is pressed against the inner surface of the oogonial wall, with the result that the appearance is presented of the oogonial cavity being entirely filled with protoplasmic contents. These contents, moreover, no longer exhibit the geometrical arrangement of the resting condition, but show instead a more or less uniformly granular condition.

If the conditions are such as to favor direct germination, one or several proturberances are now thrust through the oogonial wall. (Pl. 5, G, H.) The communications established by the fertilization tubes seem to serve an important rôle as channels of egress, as the granular germ processes can frequently be clearly seen passing through them into the antheridial cavity and thence emerging by a perforation through the antheridial wall; and owing to the collapse of the empty antheridia and difficulties in observation, instances of such utilization of these aperatures are very probably even more numerous than could be definitely established. In many cases the germ tube immediately gives rise to a number of branches, usually aggregating about a dozen, which thus occur in rather crowded, bristling arrangement not at all typical of mycelial ramification in the species (Pl. 5, L, K); in other cases branching is delayed until the germ tube has attained considerable length, and the sort of ramification then exhibited is altogether comparable to that shown by the thallus of the fungus generally. (Pl. 5, I, J.) Manifestly the conditions here represented correspond to those found in the two organisms investigated by DeBary and Kasanowsky, respectively. In appearance the resultant structures bear some similarity to those produced by the two types of direct germination of, for example, the sporangia of certain species of Phytophthora. They may, indeed, plausibly be explained in the same way—vegetative development of the oospore as a single energid, on the one hand, as contrasted with the development of multiple energids resulting from division processes incident to abortive zoospore production, on the other.

When an oospore germinates by the production of zoospores, a single filament is produced which ceases elongation after attaining a length varying from 8 to 12 times the diameter of the oogonium. (Pl. 5, E.) The germ hypha regularly decreases in width toward the tip, the distal portion usually measuring about 4 $\mu$ in this dimension, or somewhat less than one-half the diameter of the basal portion. At this stage, when only approximately half of the oogonial contents have passed into the germ hypha, zoospore formation is initiated. In the germ hypha the process shows no departure from the usual course that has been described so frequently in the filamentous vegetative sporangia of other members of the genus, yielding from 6 to 10 cylindrical portions of protoplasm connected, at least for a time, by a greatly attenuated strand. (Pl. 5, A.) It appears probable that development is in the main acropetal, the divisions delimiting the two or three most distal portions having been observed to be initiated after the separation of the basal portions had been effected, the last division of all setting off the terminal portion. Within the oogonial wall the residual material which has become concentrated in a subspherical mass near the orifice of the germ hypha undergoes similar cleavage, as evidenced by its segregation into lumps that become increasingly distinct and after some mildly writhing movements assume individuality as independent subspherical protoplasmic masses. (Pl. 5, A.) Suddenly the tip of the germ hypha gives way and the protoplasmic masses escape one by one in rapid succession, each rounding up and encysting near the orifice. An interesting feature in the evacuation of the germ sporangium is that the globose bodies within the oogonium enter the germ hypha at the base to replace distal ones by streaming through the small aperture in the oogonial wall and assuming the cylindrical form of the filament. In the course of about 10 seconds the entire apparatus is emptied. (Pl. 5, B.) The discharged spores are scattered loosely about near the mouth of the germ hypha or collected in whole or in part in a loose irregular aggregation. (Pl. 5, C, D, and F.) They number generally from 13 to 18 (most frequently 15), depending apparently somewhat on the size of the oospore and the proportion of oversized individuals capable of producing one compound or two normal motile forms.

While sporangial germination of the oospore does not appear to have been recorded hitherto for any species of Aphanomyces, it may be mentioned that a somewhat analogous development was noted by Sorokine (*16*) in the germination of globose bodies belonging to *A. stellatus*, which he designated as conidia. An extensive rupture in

the thick wall of these bodies through which the germ tube made its exit and their considerably inferior length provide differences in detail. Direct germination by a germ tube was also described for these conidia, the resulting hyphae being of the more remotely ramifying type, generally characteristic of the growing mycelium.

### ASEXUAL REPRODUCTION

It is presumably altogether safe to take for granted that the sexual spores developed in the tissues of the diseased host constitute the regular resting bodies of the fungus, by the germination of which the parasite is reestablished in successive seasons. That by the production of germ sporangia they are also the chief means by which the fungus extends its distribution in the soil, appears at least very probable, although, as is well known, dissemination of the aquatic members of the genus is effected by zoospores produced in sporangia of mycelial origin. For when infected pea tissue containing an abundance of the mycelium of the parasite is placed in water no extramatrical development takes place, the organism thus differing considerably in behavior from the amphibious species of Pythium or Phytophthora, for example, which are frequently found in similar relationship, as well as from the congeneric form reported by Peters (*13*, *14*) as causing root rot of sugar beets in Germany. The pea parasite, however, continues in its development of sexual spores apparently uninterrupted. But even if zoospores could be produced by such means it is not certain, in view of the brief time elapsing between full mycelial development and the initiation of sexual stages, that extensive zoospore formation from mycelial elements could frequently be expected in nature.

On the other hand, in artificial culture, the production of zoospores from the ordinary filamentous sporangia characteristic of the genus can be induced, and that in exceedingly great profusion. Following the well-known methods for cultivating aquatic forms, the parasite was grown in pea decoction made by adding from 8 to 10 freshly shelled peas to 100 cc. distilled water in an Erlenmeyer flask and sterilizing by autoclaving or intermittent steaming. Altogether satisfactory results were also obtained by the use of canned peas with some of the liquor in which they were obtained, as well as by employing about twice the number of dried peas to the same quantity of water. Within three or four days at ordinary temperatures an extensive submerged mycelium was produced, appearing in the liquid medium as a translucent nebulous mat. The whole growth was now transferred to a deep Petri dish, the peas removed, and the mycelium washed several times at intervals of about 15 minutes with changes of sterile water. For convenience in examination it was found desirable the last time to add only enough water to keep the mat submerged.

With young thalli at a temperature of about 20° C., evacuation of the sporangial filaments was found to begin about six to seven hours after washing was completed. As in the case of the germ sporangia, the internal developments follow the course described for congeneric species by other writers. It may not be superfluous however, to discuss certain matters which perhaps have not received adequate treatment hitherto, or which involve points in regard to which the fungus under consideration would appear to be at variance with its aquatic congeners.

For example, in the writings of most authors the distinction is made between vegetative hyphae and sporangia. While these structures are invariably said to be similar to each other in external morphology, the impression is conveyed that specialization in perhaps less obvious characteristics nevertheless obtains. Such a supposition, while conserving the analogy to other Saprolegniaceae, finds little support in the behavior of young vigorous thalli

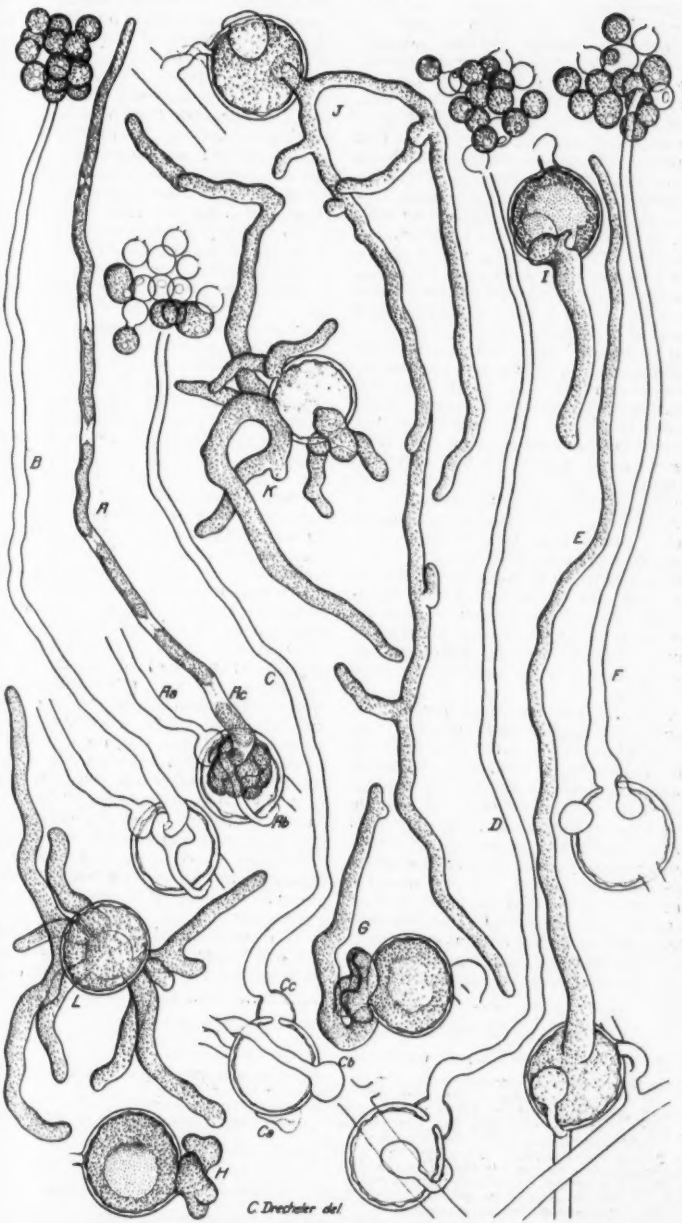
### EXPLANATORY LEGEND FOR PLATE 5

Oospores of *Aphanomyces euteiches* from 15 day old hard cornmeal agar cultures germinating in Van Tieghem preparations. × 470

A.—Germ sporangium 30 seconds before discharge
B.—Same sporangium 5 minutes later, after evacuation
C, D.—Evacuated germ sporangia showing utilization by germ hypha of aperture in oogonial wall produced by antheridium
E.—Oospore with single germ tube previous to separation of contents
F.—Evacuated germ sporangium
G to J.—Direct germination of oospores by production of hypha with ordinary type of branching
K, L.—Direct germination of oospore with close branching immediately after emergence of germ tubes

Root Rot of Peas Caused by Aphanomyces Euteiches

Plate 5




(For explanatory legend see p. 304)

subjected to the treatment outlined above. Under favorable conditions almost the entire thallus appears to become involved in sporogenesis, the individual sporangia often consisting of axial filaments from 1 to 2 mm. long and bearing from 6 to 10 well-developed branches. (Pl. 6, A.) The numbers of spores discharged from such extensive sporangial units is naturally very considerable, not infrequently running up to 300 or 400. (Pl. 6, *Bc.*) The individual sporangia are set off from adjacent sporangia, or still undifferentiated hyphae by partitions which may be plane or somewhat curved and are often inserted at the origin of a branch. (Pl. 6, A, D.) A vast number of zoospores may thus be produced in the course of a few hours, estimates made on such material often reaching several hundred thousand. It may be mentioned that the amount of growth observable after the washing away of the nutrient material is quite negligible, the possibility of extensive proliferation of new filaments originating as specialized organs being thus largely precluded. Nor do the sporangial units of such material differ in abundance of branching from the mycelium of the vegetative thallus in an actively growing condition, in spite of the customary characterization of the sporangia of congeneric forms as simple or rarely branching.

A distinction between hypha and sporangium might more plausibly be drawn when older material is used for the production of zoospores. Staling effects are here manifested in the degeneration of the contents of a large proportion of the hyphae. Sporogenesis is never prompt, a period of 48 hours usually elapsing before any considerable discharge occurs. The reason for such delay is evident on examination, when it will be found that the old hyphae are not functioning as sporangia; that these have, in fact, become evacuated, the contents having apparently been utilized in the production of new filaments in the central portions as well as at the margins of the thallus; and that sporogenesis is localized in the newly proliferated filaments, which indeed, show relatively little branching. Manifestly the potentiality of serving as sporangium is not limited to hyphae arising as specialized organs; but is inherent in any vigorous hyphae when given the necessary conditions. It may be remarked that Rothert (*15*) in his study of a congeneric form came to an entirely similar opinion in regard to this phase of reproduction.

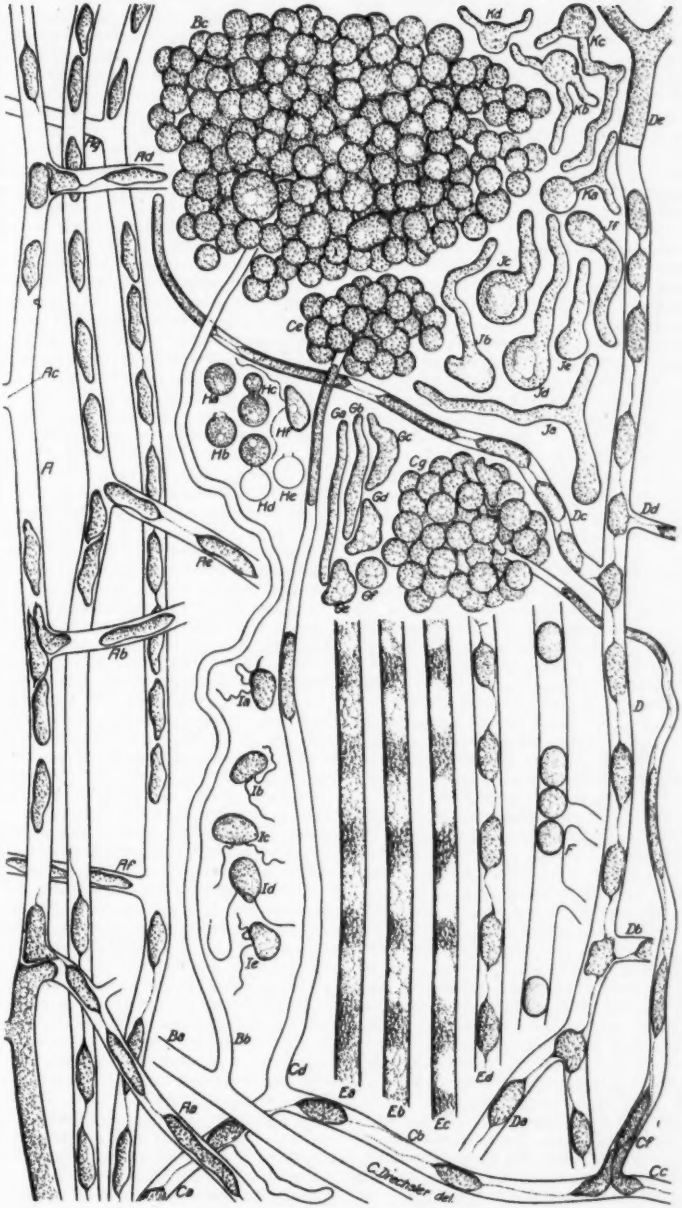
While the hyphae of the pea parasite in liquid culture, as within the host cell, tend toward evenness in diameter, pronounced local irregularities in respect to this dimension not being characteristic of the fungus, the terminal mycelial branches generally exhibit gradual attenuation toward the tip, the apical portion generally measuring 4 $\mu$, or, more rarely, somewhat less. Evacuation of the individual sporangia very regularly takes place through these attenuated branches. As the protoplasmic masses pass from the larger hyphae into the constricted region they become considerably elongated and move at a proportionately increased speed. Thus a cylindrical mass 10 to 12 $\mu$ in length, occupying the lumen of a filament 9 $\mu$ wide and moving at a speed of about 35 $\mu$ a second, on reaching the distal portion of the evacuation hyphae will be found measuring 30 to 50 $\mu$ in length and moving more than 100 $\mu$ a second. In a few instances a considerable part of the discharge tube was found reduced to a diameter scarcely exceeding 3 $\mu$. Discharge here offered the remarkable spectacle of zoospores drawn out into threadlike bodies, about 2.5 $\mu$ in diameter and 70 to 90 $\mu$ long, speeding along at the rate of approximately 300 $\mu$ per second.

The discharge of the zoospores from the freely branching sporangium presents an interesting complication. At

EXPLANATORY LEGEND FOR PLATE 6

Stages in asexual reproduction of *Aphanomyces euteiches*. ×470

A.—Portion of extensive sporangium drawn in three contiguous sections bearing six successive branches. *ab–g*, evacuation of zoospores arrested after proceeding for some time
B.—*a*, Portion of sporangial unit with aggregation of approximately 350 zoospores
C.—*a* to *c*, Portion of sporangial unit, evacuated at beginning only through branch *cd*, then through both *cd* and *cf*, and finally only through *cf*
D.—Portion of sporangium 15 seconds previous to discharge through branch *dc*
E.—*a* to *d*, Successive stages in the conversion of hyphal contents into zoospores ready for discharge
F.—Zoospores rounded up within sporangial wall
G.—*a* to *f*, Successive stages in rounding up of cylindrical protoplasts, at intervals of 2 seconds
H.—*a* to *f*, Evacuation of encysted zoospore
I.—*a* to *e*, Motile zoospores treated with osmic acid and gentian violet, showing length and insertion of flagella
J.—*a* to *f*, Direct germination of encysted zoospores
K.—*a* to *d*, Germination of zoospores after swarming and second rounding up




(For explanatory legend see p. 306)

each juncture of axial filament and branch two of the elements deliver their contents into the third. Where evacuation is not too rapid, the delivery alternates in a more or less orderly manner, one or several zoospores from one arm being followed by one or several zoospores from the other. When discharge is more rapid, some degree of disorder usually results, a number of zoospores from both sources often being squeezed into the efferent element so compactly that they may momentarily appear as a single protoplasmic mass (pl. 6, *Cf*) which further along in the course of the filament tends to become separated into its components.

As in other members of the genus Aphanomyces, the changes (pl. 6, *Ea*, C), resulting in the division of the mycelial contents into zoospores, leaves the individual protoplasmic masses connected by a delicate strand of tenuous material. (Pl. 6, Ed, D.) This strand can usually be seen without difficulty after evacuation has started, even when it has become further attenuated by lengthening of the intervals between successive zoospores. However, beyond the first juncture of two sporogenous elements the strands uniting the zoospores contributed by each of the elements are pressed against the confining mycelial wall by the interpolated zoospores contributed by the other, with the result that their continuity, if not actually destroyed, becomes at least very difficult to establish. After several junctures have been passed, so that the moving file of zoospores represents, perhaps, more than half a dozen interpolated series, it is certainly not possbile to make out a corresponding number of strands in the intervals. Usually only one or two can be made out in any particular gap, depending upon whether a strand can be demonstrated for one or for both of the two successive zoospores. (Pl. 6, D.) Rothert (*15*) in his study of an unnamed species of Aphanomyces believed that the strand persisted even where none was visible; that the fact of the escaping protoplasts being pointed at the ends indicated clearly enough the presence of a connecting medium capable of exerting a pull. To the traction exerted by the distal zoospores he assigned some importance in accomplishing the evacuation of the proximal ones. In the pea parasite, however, the zoospore while passing through the evacuation hypha are not always pointed at the ends, the anterior end especially being frequently well rounded as if no distorting pull were present.

A curious feature exhibited by the root-rot fungus which does not seem to have been recorded hitherto for any congeneric form is the discharge of the zoospores from a sporangial unit through plural evacuation hyphae. In the extensive units characteristic of vigorous young thalli converted to reproductive purposes, three or even four evacuation tubes have been found; and sometimes two of these may be close enough together that they can be observed simultaneously. Such an instance is represented in Plate 6, C. Evacuation here began through branch *Cd* and had proceeded briskly for about 15 seconds when the tip of branch *Cf* also yielded. For a number of seconds discharge occurred simultaneously with about equal rapidity from both tubes, the element *Ca* supplying tube *Cd*, while *Cf* was supplied from the element *Cc*. Soon discharge through *Cd* came to a standstill, and the element *Ca* contributed its zoospores through the intermediate portion *Cb* into *Cf*, the latter then being fed, as *Cd* had been previously, from both directions. A number of zoospores in the portion *Cb*, that had originally come from *Cc* and seemed bound at the time to emerge through hypha *Cd*, thus reversed their direction and were discharged through branch *Cf*. In other cases evacuation through two tubes was observed to take place simultaneously through both, now through one, now through the other, in repeated and apparently haphazard alternations. The entire process, with its reversals in direction resulting in the discharge of successive zoospores through separate evacuation branches, failed to suggest any considerable effectiveness of visible and possibly invisible connecting strands in determining the course of any individual zoospore.

On emerging from the mouth of the sporangium the spores are cylindrical in shape, straight or slightly curved. (Pl. 6, *Ga*.) Immediately, however, they begin to shorten up, and after passing through increasingly thick allantoid phases (pl. 6, *Gb* to *e*), appear at the end of about 10 seconds as perfectly spherical masses (pl. 6, *Gf*, although a certain proportion of irregular oversized individuals may usually be found. (Pl. 6 *Bc*, *Ce*, *Cg*.) The secretion of a thin peripheral wall follows very shortly. While in certain species of Aphanomyces the quiescent zoospores arrange themselves in a very regular hollow sphere having some considerable degree of coherence, in the form under consideration these bodies show little tendency toward definite orientation and relatively little co-

herence. They seem merely to accumulate promiscuously at the mouth of the sporangium in an irregular lump (pl. 6, *Bc*, *Ce*, and *Cg*), or where smaller numbers are concerned each individual may remain alone.

The development of motile zoospores from these encysted bodies takes place after a period varying in some observed instances from 1½ to 2½ hours. A papilla makes its appearance on the cyst, measuring between one-fourth to one-third the diameter of the latter. (Pl. 6, *Hb.*) At first scarcely discernible, it develops visibly in the course of several minutes, until it appears as a hemispherical protuberance. Suddenly the granular protoplasmic contents begin streaming into the papilla, the tip of which thus becomes inflated into a spherical vesicle. (Pl. 6, *Hc.*) Streaming is completed within approximately 10 seconds, the entire contents having then passed from the cyst into the vesicle. (Pl. 6, *He.*) The contour of the latter is not tangent to that of the empty cyst envelope, as these two structures are separated by a cylindrical isthmus, about 1 $\mu$ long, composed of the wall of the papilla. The whole process appears altogether analogous to the discharge of the sporangium of Pythium on a smaller scale, and, as in that genus, the discharged protoplast, after increasing distortional movements, finally develops motility and swims away, not as a number of zoospores, to be sure, but as a single zoospore, somewhat pointed at one end, with two cilia inserted laterally. (Pl. 6, *Hf.*) In material killed with osmic acid and stained with gentian violet, the flagella appear as thread-like structures approximately twice the length of the zoospore, one being somewhat longer than the other but the difference not being pronounced. (Pl. 6, *Ia* to *e.*)

After swimming about for a variable period the zoospore, as in other members of the Saprolegniaceae, finally come to rest and round up. Under favorable conditions they germinate, producing from one to three germ tubes (pl. 6, *Ka* to *d*) capable of extensive development into a mycelium, either in artificial culture or in the tissues of a new host plant.

It may be superfluous to call attention to rather usual irregularities in development, cited frequently in the literature pertaining to related organisms. Among these might be mentioned the failure of the zoospores to escape from the sporangium (pl. 6, F) and their germination within the filament; the production of compound zoospores exhibiting two sets of flagella with erratic ineffectual movement, resulting from the germination of oversized and evidently dienergid cysts; and direct germination, without swarming, of encysted forms by the production of a single rather broad germ tube. (Pl. 6, *Ja* to *f.*)

### TAXONOMY OF PARASITE

As suggested in another connection, it is not improbable that the parasite under consideration may have been observed by previous workers investigating the troubles affecting peas. Wittmack (*19*), in Germany, attached the binomial *Pythium sadebeckianum* to a fungus found occurring as oospores in the roots of peas, the presence of which was evidently associated with symptoms having some similarity to those described in this paper. Chiefly because the diameter of oogonia, as given by this author, —32 $\mu$ is in excess over those of any species of Pythium commonly attacking higher plants, students of the latter genus have been at a loss as to the identity of the German fungus. Judging from the widespread occurrence of the root rot parasite in diseased pea roots in the United States, it is at least likely that Wittmack, who apparently never observed sporangia in his material, may have assigned his fungus to the wrong genus. For quite similar reasons the same uncertainty attaches to Clinton's (*2*, p. 450–453) provisional identification of oospores observed during recent years in diseased pea roots in Connecticut as the oospores of *Phytophthora cactorum*. The identity of the forms observed by these writers with the fungus discussed in this paper at present can merely be suggested as a very fair possibility.

In this connection it may not be amiss to call attention to the somewhat unusual parasitic character of the rootrot organism when viewed in its taxonomic relation. While the Saprolegniaceae include a number of parasites affecting fish, only a few reports can be found of any members of the family attacking species of higher plants. In 1912 Sawada[5] published a paper containing a detailed study of *Achlya prolifera* (Nees) DeBary, as the cause of a rice seedling

[5] SAWADA, K. INVESTIGATION OF THE PADDY SEEDLING DECAY IN FORMOSA. Formosa Agr. Exp. Sta. Spec. Bul. 3, 84 p., illus. 1912. [In Japanese.] Brief statement of content in English, by W. H. Weston in Ann. Bot. 37: 347–348, 1923. Author's unpublished English résumé revised by W. H. Weston, deposited in Office of Cereal Investigations, Bureau of Plant Industry, Washington, D. C.

decay in Formosa. Nemec (*12*) in 1913 described from Prague as the type of a new genus an interesting fungus causing swellings on the roots of *Salix purpurea* under the binmoia *Jaraia salicis*. It is worthy of note that the fungi in these two instances effected their parasitism under conditions substantially aquatic, the willow plants attacked by *Jaraia salicis* being cultivated at the time in tap water in the greenhouse, while the destruction observed by Sawada presumably occurred while the host was kept flooded.

Within the genus Aphanomyces itself the tendency toward parasitism is moderately pronounced. Two of the eight described species, *A. phycophilus* DeBary and *A. norvegicus* Wille, attack species of Spirogyra and Zygnema (*1; 18*); two other members of the genus according to Coker, *A. stellatus* DeBary and *A. parasiticus* Coker (*3*), attack Achlya, and the same author discusses a variety of *A. laevis* DeBary that was found growing parasitically on diatoms and desmids. In these instances the hosts represent aquatic lower forms attacked under thoroughly aquatic conditions. The only account of a member of the genus, and, indeed, of the family, as far as the writers have been able to determine, attacking one of the higher plants under ordinary terrestrial conditions is contained in the report by Peters (*14*) of a form of Aphanomyces identified by him as *A. laevis*, as one of the three widely prevalent parasites responsible for root blight (Wurzelbrand) of sugar beets in Germany. Peters' publication is of particular interest, although in some pathological features the disease he investigated differs from the trouble affecting the subterranean parts of peas.

In considering the taxonomic disposition of the pea fungus, moreover, *Aphanomyces laevis* represents one of the two species deserving of special attention, the other being *A. helicoides* v. Minden. The remaining six congeneric forms are characterized by the presence on the oogonium of spines or of tuberculate irregularities, whereas the oogonia of the pea parasite, like those of the two species designated, are entirely smooth on the exterior. According to its author (*11*), *A. helicoides* is very similar to *A. laevis*, being distinguished chiefly by a strong tendency in the antheridial branches to wind about the oogonium, or about ordinary hyphae, or even about other antheridial branches, in close helicoid turns. Certain of Kasanowsky's drawings (*10:* Taf. X, fig. 1) represent some such condition, making it seem probable that this investigator was dealing with a type more nearly resembling the Swiss form than the one originally described by DeBary. Coker seems inclined to support Von Minden's own doubts concerning the validity of the helicoid habit as a specific distinction on the ground that a strong tendency toward such habit was exhibited by the typical form of *A. laevis*. Whatever disposition may finally be taken with reference to *A. helicoides* is, however, of minor concern here, for, as has been previously pointed out, no distinct tendency toward spiral growth has ever been found expressed in any of the cultures of the pea organism the writers have studied, although the branches bearing the sexual organs may be more or less involved after a more promiscuous fashion.

When the organism responsible for root rot of peas is compared with the typical aquatic form of *Aphanomyces laevis*, as revealed in the literature, a considerable measure of agreement becomes evident. The dimensions and interrelations of oogonium and antheridium and of the branches supporting them, as well as the dimensions of the oospores, correspond quite well in the two plants. A fair, even if not altogether perfect, agreement is evident in regard to size of zoospore and diameter of mycelium. The potentiality of the entire thallus to serve reproductive purposes by division into extensive branching portions functioning as individual sporangia; the discharge of the latter through one or several hyphae tapering regularly toward the tip to a diameter inferior to the diameter of the mycelium generally; the ready germination of the oospores, and that without resting period or application of special treatment—these features point to a distinction between the root parasite and the aquatic form, although it remains doubtful to what extent absence of their mention in the literature pertaining to the latter form is attributable to actual difference in its behavior or to shortcomings in our knowledge concerning it.

With regard to another character—namely, the thickness of the oogonial wall—it becomes almost impossible to entertain similar doubts. The accounts of *Aphanomyces laevis* given by DeBary, Humphrey (*5*), Kasanowsky, and Coker contain no reference to this feature, yet it is altogether unlikely that any of these investigators could have seen a structure of such extraordinary thickness without in some way referring to it. Indeed, on the contrary, Coker in his definition of the genus

Aphanomyces, recognizes a thin unpitted oogonial wall as a generic characteristic, presumably common to all species; and all of the authors mentioned figure the oogonial envelope of *A. laevis* as a thin membrane, not to be compared with even the least indurated of the homologous structures belonging to the parasite on pea roots.

In this connection it may be mentioned that the writers have cultivated an aquatic species of Aphanomyces with smooth oogonia, obtained through the courtesy of J. A. Lounsbury, who isolated it from water of Lake Mendota, where apparently it occurs in some abundance. This fungus, which as far as could be determined answers to the description of *A. laevis*, very obviously is not identical with the parasite affecting peas, developing a heavy matted, submerged growth on various agar media entirely unlike the arachnoid growth characteristic of the terrestrial organism. The oogonia are produced much more sparingly and always with the thin membranous wall made familiar by the figures of various writers. As the different isolations made from diseased peas compared with one another show unusual uniformity with reference to morphological characteristics, as well with reference to rapidity and character of growth on a variety of substrata, their incorporation in the same species with a form so evidently dissimilar as the aquatic plant could scarcely contribute to taxonomic clarity.

The identity of Peters' beet parasite remains problematical. This author undoubtedly was right in concluding that the measurements of his fungus, while somewhat different from those of *Aphanomyces laevis*, did not, in themselves, justify its recognition as a species distinct from the latter. It is interesting to note that he observed very marked variability in thickness of both oogonial and oospore walls, and as the thickness of these structures fluctuated together he believed that the fluctuations were contingent on the same environmental conditions affecting both. Thus in material containing oogonia so thin-walled that they were found collapsed, the oospores borne in them were provided with walls *only* 3 $\mu$ thick; while in other material oogonia with walls 1.5 $\mu$ thick contained oospores of which the envelope measured 5 to 6 $\mu$ in thickness. If the measurements of the oospore wall as given represent measurements of normal material, Peters' fungus would undoubtedly constitute a species different from any species of Aphanomyces hitherto described, as well as from the one described in this paper. However, grounds for suspecting that this is not the case are not wanting. In the pea parasite, for example, degenerate conditions of the sexual apparatus are far from rare even on the most favorable substratum like hard corn-meal agar; when less favorable media are used (potato or carrot decoction with 1.5 per cent agar, and also pea decoction in the absence of solid particles) degenerate conditions represent the overwhelming rule. The contents of the oogonium may become lumpy and degenerate before an oosphere is formed, or an oosphere may be formed but degenerate without forming a wall, or a wall may be formed but the contents degenerate instead of developing the normal structure. In any case swelling of the confining membrane is a regular concomitant of protoplasmic degeneration, whether this occur in oogonium or oospore, and in the absence of normal material such pathological modification might be mistaken for normal thickening. In view of these circumstances, it is hardly possible to decide definitely as to the morphological relationship of the two congeneric forms, both terrestrial in habitat and parasitic on a phanerogamic host.

It may also be stated that in the study of the pea organism structures involved in degenerative changes were not given weight; that the peculiarities of the oogonial wall submitted as normal were observed regularly in nature, as well as in culture, in individual female organs containing oospores provided with a wall of moderately and practically unvarying thickness containing a large, homogeneous, slightly eccentric ("subcentric" in the phraseology used by Coker) structure, surrounded by concentrically arranged granular-appearing bodies. The parasite, it is believed, may best be regarded as a new species. Because of the distinctive character of the oogonial envelope, the specific name *euteiches* is suggested.

## TECHNICAL DESCRIPTION

### APHANOMYCES EUTEICHES Drechsler n. sp.

Parasitic on the subterranean parts of cultivated peas (*Pisum sativum* L.), causing a destructive stem and rootrot capable of affecting the growing host at all ages.

Hyphae hyaline, branching at moderate intervals (20 to 150 $\mu$) at angles approaching a right angle; 4 to 10 $\mu$ in diameter, the individual filaments not

abruptly varying in width; occurring in nature within cortical cells of host, in nutrient solutions as extensive nebulous translucent mycelia.

Sporangia in artificial culture arising by conversion of extensive portions of vegetative mycelium delimited by one or more septa; often including many ramifications; discharging through one or several (up to four) tapering branches, the distal portions of which measure usually approximately 4 $\mu$.

Zoospores cylindrical, in escaping from evacuation branches becoming attenuated to vermiform bodies, usually 3.5 $\mu$ in diameter by 30 to 50 $\mu$ in length; forming spherical cysts at mouth of sporangium, measuring usually 8 to 11 $\mu$ in diameter, rarely up to 16 $\mu$; diplanetic, the empty spherical wall being distinguished by a protruding evacuation tube 1 $\mu$ long by 2.5 to 3 $\mu$ in diameter.

Oogonium generally, if not always, terminal on a short lateral branch, from which it is delimited by a partition sometimes present as a simple septum, at other times as a columella-like structure protruding into the oogonial cavity; subspherical, measuring usually 25 to 35 $\mu$ in diameter; when mature exhibiting a heavy peripheral wall with smooth outer contour and sinuous inner contour, hence of irregular thickness, this dimension varying between 1 to 5 $\mu$, generally between 1 to 2.5 $\mu$.

Antheridia typically of diclinous origin, borne on a stalk frequently involved with the oogonial stalk, and often branching once or several times; measuring 8 to 10 $\mu$ in diameter by 15 to 18 $\mu$ in length, or when considerably larger often more conspicuously arched, somewhat lobulate, and becoming compound by the insertion of transverse septa.

Oospores subspherical or more rarely ellipsoidal owing to intruding columella-like septum; 18 to 25 $\mu$ (generally 20 to 23 $\mu$) in diameter; provided with a wall of uniform thickness, this dimension varying between 1.2 and 1.8 $\mu$ (generally 1.5 $\mu$); slightly eccentric in internal structure ("subcentric"); germinating without protracted resting period either directly by 1 to 3 germ hyphae or by production of a single unbranched sporangial filament usually 200 to 350 $\mu$ in length, in the latter event producing generally 13 to 18 zoospores, approximately half of which are delimited within oospore wall.

Collected repeatedly in diseased peas in Maryland, New York, Ohio, Indiana, Michigan, Illinois, Wisconsin, Montana, Idaho, Utah, and California.

## PHYSIOLOGY OF PARASITE

### GROWTH OF THE FUNGUS ON CULTURE MEDIA

The fungus has been grown on several of the common culture media, on all of which it produces more or less of a sparse white surface growth with little aerial mycelium. A few imperfectly formed oospores are usually found in older cultures on most media. However, on corn-meal agar, while the mycelial growth is rather less than on most substrata tried, there is a moderately abundant production of oospores which are capable of germination soon after they are formed. For this reason cultures on this substratum retain their vitality indefinitely, while those on other media soon perish. Semisolid media, such as cornmeal or oatmeal with varying amounts of water added also give good growth, but the oospores are less perfectly formed as the medium becomes more moist. The most useful liquid medium that has been found for the production of mycelium and sporangia is a pea decoction made by adding 10 to 20 peas, preferably of a wrinkled variety, to about 75 cc. of water in a flask. This liquid remains clear after sterilization in the autoclave, and mycelium growing in it can readily be washed free from all nutrient material. The fungus grows as a submerged tuft in this medium until it reaches the surface, over which it spreads as a mat. In about 7 to 10 days at room temperature oospores begin to form, and soon after most of the mycelium is found empty and dead.

### EFFECT OF TEMPERATURE UPON THE DEVELOPMENT OF THE FUNGUS

GROWTH OF MYCELIUM.—The fact that this disease develops early in the spring indicates that the fungus must be able to thrive at comparatively low soil temperatures. In an experimental determination of the thermal limits within which the fungus will grow a semiliquid medium, composed of 1 part by weight of corn meal with 12 parts of water, was used. The growth of the fungus in the surface of this gelatinous material is so inconspicuous that small increments of growth can not be measured accurately, and while the limits of growth were determined, the optimum could only be roughly estimated. In cultures held at a series of constant temperatures in incubators vigorous growth occurred at 34° C., but no growth was observed at 37°. At the lower end of the series no growth was observed after six days at 8° to 10°,

while a growth of 1.5 cm. occurred at 9° to 11° in the same time. The optimum temperature for mycelial growth appeared to be between 15° and 34°.

FORMATION OF ZOOSPORES.—The temperatures at which zoospores were discharged from sporangia and became motile was determined as follows: A culture upon oatmeal mush six days old was washed as free as possible from its substratum, cut into fragments, and placed in sterile water in watch glasses which were distributed in incubators at a series of temperatures. The results are presented in Table I.

drops of sterile water to which spore-bearing mycelium with as little substratum as possible has been transferred. Germination has been secured at temperatures from 6.5° to 31° C. Germination with the production of zoospores occurs in 24 to 48 hours at 14° to 28°, a range that may be regarded as an optimum. Germination with the production of a few zoospores has been secured at a temperature as low as 9° to 10° in four days, while germ tubes which did not discharge zoospores developed in the same time at 6° to 7°. Thus the oospores germinate in practically the

TABLE I.—*The effect of temperature upon the formation and motility of zoospores of Aphanomyces euteiches and the germination of zoospores*

| Temperature °C. | Time in hours | | | | | |
|---|---|---|---|---|---|---|
| | 18 | 25 | 42 | 49 | 73 | 97 |
| 33-35 | | Groups. | Groups. | Disintegrating. | | |
| 21-22 | Groups. | Groups. | Groups. Zoospores. | Inactive. | | |
| 19.5-21 | Groups. | Groups. Zoospores. | Groups. Zoospores. | Inactive. | | |
| 15-16 | Groups. | Groups. Zoospores. | Groups. Zoospores. | Groups. Zoospores. | Groups. Zoospores. | Inactive. |
| 13-14 | | Groups. | Groups. Zoospores. | Groups. Zoospores. | Groups. Zoospores. | Inactive. |
| 9-11 | | | Groups. | Groups. Zoospores. | Groups. Zoospores. Groups. | Zoospores. Groups. |
| 8-9.5 | | | | | | |
| 5-6.5 | | | | | | |

NOTE.—The word "Groups" indicates the presence of groups of discharged encysted zoospores at the time of observation, and "zoospores" the presence of motile conditions.

GERMINATION OF OOSPORES.—The determination of the effect of temperature upon the germination of oospores has been rendered difficult by reason of the fact that no dependable method has been developed whereby a quantity of mature oospores can be produced at a given time. Oospores from the roots of peas have not been seen to germinate. Frequently cultures on corn-meal agar three weeks old will germinate readily when placed in water, but sometimes only one culture among many made at the same time will respond in this way. Spores on the scant aerial mycelium which can be separated from the substratum usually germinate sparsely, while spores on or in the substratum often begin germination as soon as mature. In spite of the difficulties which have arisen from these irregularities in behavior, fragmentary records have been made which appear to delimit closely the range within which germination occurs and the optimum. Germination has been studied in hanging

entire range of temperature at which vegetative activity occurs, and the discharge of zoospores from the sporanges produced by the germination is affected by temperature in exactly the same manner as in the previous experiments where sporanges derived more remotely from oospores were used.

## PATHOGENICITY

### PENETRATION

A study of lesions in early stages occurring in the field or in greenhouse inoculations rarely reveals the first penetration of the fungus into tissue. In roots entry is accomplished without discoloration or visible indication of the fact, and later the entering mycelium, devoid of contents, is practically invisible. In the base of the stem a yellow discoloration in a few epidermal cells often indicates a point of entry. The invasion of the base of the stem is usually accomplished by the advance of the fungus upward from the root

rather than from direct entry. When peas are sterilized superficially and grown under sterile conditions in agar upon which the fungus is also growing, penetration of roots can then be found readily in thin razor sections of the root surface. The mycelium passing along the root surface turns inward at the junction of two epidermal cells and passes readily into one of these cells, whence it advances into an adjoining cell or into underlying tissue. (Pl. 3, B.) The point of entry and passage from one cell to another is not marked by any conspicuous constriction of the fungus strand. Invaded tissue softens immediately even in the absence of bacteria, indicating perhaps the production of an enzyme which softens cell walls and aids in fungal penetration.

## RELATION OF SOIL TEMPERATURE AND MOISTURE TO THE DEVELOPMENT OF THE DISEASE

### EXPERIMENTAL STUDIES

Among the many diseases of roots of plants few show in the field a greater degree of apparently erratic irregularity than the one under consideration. Even in fields which are very severely infested the disease is usually much worse in spots, and at its first appearance it occurs in irregular areas which are frequently coincident with the more moist soil, but at other times are not clearly related to any obvious soil differences. It appears that the occurrence of this disease is greatly influenced by environmental conditions. In its seasonal development temperature is undoubtedly a limiting factor, and in its local occurrence the field evidence suggests soil moisture as the more important limiting factor. In order to gain exact experimental evidence of the relations of these factors to the development of the disease, several series of plantings were made in the Wisconsin soil temperature tanks where both temperature and moisture were controlled within narrow limits. Two of these series will be reported in detail.

The soil chosen was a sandy loam from a field in which peas had never been grown, and as a further precaution against the presence of root-destroying fungi it was treated with formaldehyde a month before use and subsequently thoroughly dried until no trace of this sterilizing agent was detected. The soil was inoculated one week before planting by mixing thoroughly with small fragments of roll cultures of an isolation of this fungus which had shown very active parasitism in previous tests. The viability of the oospores in these cultures had also been previously demonstrated. The inoculated soil was packed in 6-inch cans, near the bottom of which an irrigation apparatus was arranged to permit the addition of water without flooding the surface of the soil. On November 18, 15 Alaska peas were planted at the depth of about 2½ inches in each can. Six cans were placed in each of a series of tanks, which were maintained at 12°, 18°, 24°, and 30° C., respectively. The moisture present in the soil at planting was 15 per cent of its dry weight, or about 35 per cent of the moisture-holding capacity of the soil, previously determined to be 42 per cent of its dry weight. Two of the cans in each tank were maintained at the original soil moisture, while in two others the water was increased to 60 per cent of the moisture-holding capacity of the soil, and in the remaining two cans it was raised to 80 per cent of the moisture-holding capacity.

Inoculation in this instance was so effective that soon after they had emerged from the ground a few of the plants decayed and wilted down, whereupon they were removed. At the conclusion of the series, on December 18, the remaining plants were removed from the soil, and record taken of those showing decay of the base of the stem and of the roots. (See Table II.)

From these data it appears that the fungus was restrained little if any in its parasitic activity toward peas at 30° C., which is within 3° of the upper limit of soil temperature at which peas can be induced to make an approximately normal growth. In soil with the highest moisture content the progress of the disease does not appear to be favored as much as might be expected. Since some degree of infection took place even at the lowest soil temperature maintained here, and the activity of the parasite at the lower range of temperature is of greater interest, a new series was started December 19, with the purpose of exploring more thoroughly the range of temperature which occurs in the field at the time when the disease normally appears.

In the second series the soil used before was mixed, dried somewhat, and placed in cans. The moisture in this soil was found to be 36 per cent of its moisture-holding capacity, and adjustments were made to 60 and 80 per cent to correspond with the earlier series. (See Table III.)

TABLE II.—*The pathogenicity of Aphanomyces euteiches to Alaska peas at the series of soil temperatures and in soil at the three conditions of soil moisture indicated (series of November 18, 1923)*

| Temperature in °C | 12° | | | 18° | | | 24° | | | 30° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Per cent moisture-holding capacity of soil | 35 | 60 | 80 | 35 | 60 | 80 | 35 | 60 | 80 | 35 | 60 | 80 |
| Number of plants Dec. 1 | 25 | 27 | 25 | 27 | 26 | 26 | 25 | 25 | 24 | 28 | 26 | 27 |
| Number of plants wilting and removed at date indicated: | | | | | | | | | | | | |
| Dec. 1 | | | | | | | | 1 | 2 | | | 3 |
| Dec. 2 | | | | | | | | 2 | 4 | | | 2 |
| Dec. 6 | | | | | | | 2 | 1 | 7 | | 7 | 6 |
| Dec. 8 | | | | | 1 | 2 | | 3 | 1 | | 7 | 2 |
| Dec. 13 | | | | | 1 | 2 | 8 | 5 | 4 | | 4 | 2 |
| Dec. 17 | | | | 3 | 2 | 1 | 5 | 2 | | | 2 | 1 |
| Total number of dead plants | | | | 3 | 4 | 5 | 15 | 14 | 18 | (a) | 20 | 16 |
| Number of plants with decayed stem bases Dec. 18 | 3 | 9 | 3 | 10 | 19 | 11 | 10 | 4 | (b) | 1 | 3 | 8 |
| Total number of plants infected | 3 | 9 | 3 | 13 | 23 | 16 | 25 | 18 | | 1 | 23 | 24 |
| Healthy plants, Dec. 18 | 25 | 21 | 25 | 15 | 5 | 8 | 0 | 5 | (b) | 12 | 4 | 1 |

[a] One of the two cans at this temperature and moisture developed a leak and was discarded.
[b] Data missing.

TABLE III.—*The pathogenicity of Aphanomyces euteiches to Alaska peas at the series of soil temperatures and at the three conditions of soil moisture indicated (series of December 19, 1922)*

| Temperature in ° C | 10° | | | 12° | | | 15° | | | 18° | | | 21° | | | 24° | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Per cent moisture-holding capacity of soil | 36 | 60 | 80 | 36 | 60 | 80 | 36 | 60 | 80 | 36 | 60 | 80 | 36 | 60 | 80 | 36 | 60 | 80 |
| Number of plants Jan. 5 | | | | | | | 27 | 36 | 32 | 34 | 34 | 28 | 38 | 24 | 32 | 39 | 31 | 28 |
| Number of plants wilting and removed at date indicated: | | | | | | | | | | | | | | | | | | |
| Jan. 2 | | | | | | | | | | | | 1 | | | 1 | | 1 | 3 |
| Jan. 5 | | | | | | | | | | | | | | 2 | 1 | | | |
| Jan. 9 | | | | | | | | | 1 | | | 5 | | 4 | 3 | 4 | 1 | 13 |
| Jan. 12 | | | | | | | | | 2 | | 1 | 1 | | | | 6 | | 3 |
| Jan. 19 | | | | | | | | | 3 | 5 | | 4 | | 1 | 3 | 7 | 2 | 4 |
| Total number of plants killed | | | | | | | | | 6 | 5 | 1 | 11 | | 7 | 8 | 17 | 4 | 23 |
| Number of plants diseased Jan. 30 | | 1 | 1 | 2 | 0 | 2 | 17 | 8 | 10 | 22 | 19 | 6 | 10 | 5 | 17 | 12 | 7 | 5 |
| Total number of plants infected | | 1 | 1 | 2 | 0 | 2 | 17 | 8 | 16 | 27 | 20 | 17 | 10 | 12 | 25 | 29 | 11 | 28 |
| Healthy plants Jan. 30 | | 29 | 20 | 30 | 32 | 19 | 14 | 25 | 16 | 6 | 13 | 10 | 24 | 13 | 8 | 7 | 17 | 0 |

The soil from this experiment was used for a third series in the temperature tanks, in which the method was modified for the purpose of learning whether infection occurring later in the development of the plant would produce less wilting than early infection. The soil was dried until it contained 30 per cent of its moisture-holding capacity, mixed thoroughly, and placed in cans. Peas were planted on February 10, and the entire series was kept at a soil temperature of 12° C. until February 24, when most of the plants had emerged, though not all had assumed an erect position. Temperature was adjusted to 15°, 18°, 21°, and 27°, and water was added to bring the soil up to 60 per cent of its moisture-holding capacity. New inoculation of these plants was then made by pouring a suspension of zoospores around the bases of all plants, and the surface soil was kept moist for the three following days. In five days a few plants at 27° showed infection at the surface of the soil, from which they died later; but there was so little infection at the lower temperatures that the results of this series are not

presented in detail. The paucity of infection in this experiment is not easily explained.

The data presented from the two previous series indicate clearly that though occasional infection may take place through practically the entire range of temperature at which peas will grow, infection is not abundant nor does invasion proceed rapidly at a temperature below 15° C. The optimum temperature for infection is between 15° and 30°. Within this range infection appears to be approximately uniformly abundant, but at the lower temperatures there is a retardation in rate of progress of the fungus through the plant that is even greater than the retarding effect of temperature upon root growth as it occurs under the conditions of experiment. It is probable, however, that in the field in the spring with longer days root growth is more rapid at lower temperatures than under the conditions of experiment, and if that is the case the apparent retarding effect of low temperature upon the progress of the disease in the field will be greater than is shown here.

The effect of soil moisture upon infection in these experiments was not as great as anticipated. In fact, they do not give any decisive indication that soil moisture within the limits used in these experiments is a factor at all in determining the amount of infection. Although infected plants in the more moist soil uniformly begin to die a little earlier than those in drier soil, the number is in most cases approximately equal.

Further consideration of these experimental data may serve to bring out more clearly their limitations when an attempt is made to interpret field experience in their light. In these experiments it may be assumed that an approximately equal amount of viable fungus mycelium was brought in contact with the root systems of plants. The number of infections obtained is an indication of the ability of the fungus to infect under these conditions. It is possible that in the field soil moisture exerts an important effect, not only upon infection, but upon the survival of the fungus from year to year. The favorable effect of wet soil upon the disease may be due in large part, if not wholly, to the favorable environment which moisture provides for the fungus in its resting condition as oospores, or in a possible saprophytic life in the soil. Such an effect of soil moisture increasing the active vegetative growth of the fungus in the field may account for the association of disease with wet soil, rather than any effect of moisture upon the penetration of the plant by the parasite.

These experimental data should be compared carefully with observations of the development of the disease in the field on infested land to determine whether the inferences which may be drawn from the experiments are supported by practical experience. Unfortunately, there is but a limited amount of exact field observation which will serve for comparison. The development of the disease in experimental plats at Madison, Wis., on ground thoroughly infested with the fungus has been followed more closely than elsewhere. Planting in this plat was made in 1922 and 1923 on the same date, April 28. In 1923, the roots from these plants remained free from any trace of disease until May 30, when a very few rootlets showing typical decay due to Aphanomyces were found. On June 5 the entire root systems of these plants had begun to decay from a great number of infections distributed from the surface of the ground to the deepest roots. In this brief space of time abundant oogonia and a few mature oospores were formed.

This sudden appearance of disease followed promptly after the first favorable period of temperature and moisture that had occurred since the crop was planted. (Fig. 1.) The month of May of that year was exceedingly dry. The only rains sufficient to wet the soil for a brief period had fallen on May 15, 19, and June 2. The first rain occurred when the plants were emerging from the ground, and was followed by cold weather, the soil temperature[6] at a depth of 2 inches falling at night as low as 1° to 7° C. The second rain on the 19th was followed by weather hardly warmer, the mean soil temperature for the next five days ranging from 9° to 13.3°, a temperature at which oospores would germinate very slowly

The temperature rose very slowly after this date until June 2, when a heavy thunder storm gave a precipitation of 0.92 inch, wetting the soil in the plats to a depth of about 5 inches. After this day the minimum soil temperature did not fall below 15° C., and the mean daily soil temperature rose in the following five days from 17.5° to 20.5°. This coincidence of moisture with high soil temperature furnished optimum conditions for the

[6] The writer is indebted to J. G. Dickson for the use of soil temperature records quoted here.

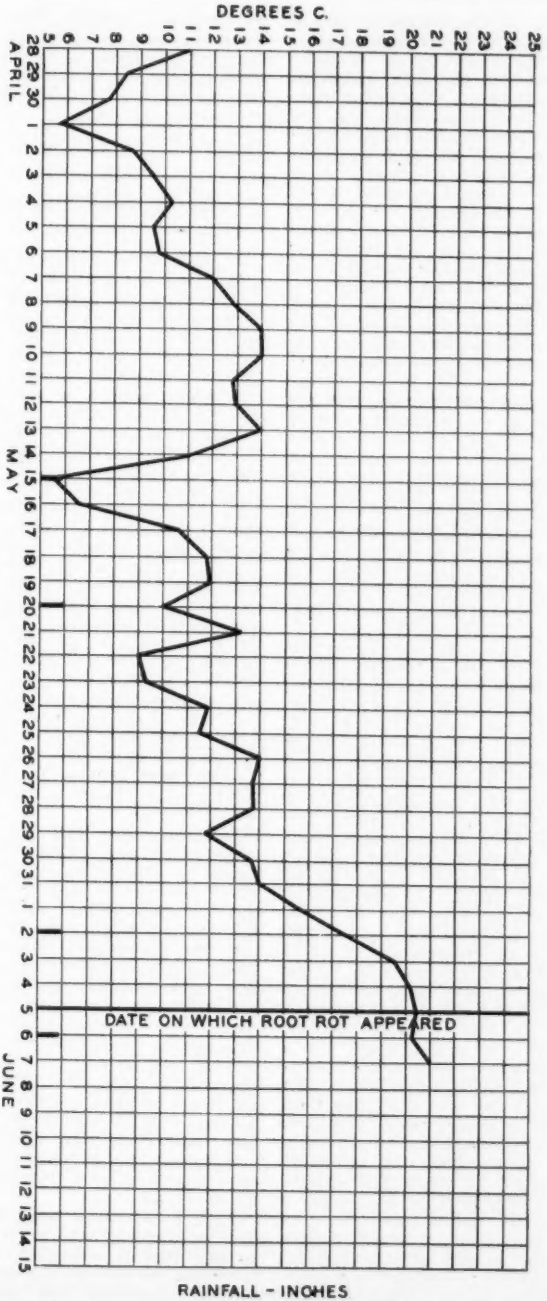



FIG. 1.—Average daily soil temperature in experimental plats at a depth of 2 inches and precipitation at Madison, Wis., from April 28 to June 15, 1923.

development of the disease which appeared with such remarkable suddenness. In fact, the period of time elapsing between the rain and such extensive invasion of the plant was so brief that it seems unlikely that the rain started the fungus to active vegetative growth from oospores, but rather that it had achieved some development during the period of dry weather. A heavy rain on June 6 with continued high soil temperature maintained favorable conditions for the development of the disease for a brief time until it was checked by returning drought, which lasted until the end of the growing period of the crop. It may be added that the shower of June 2 was local, not extending to the entire pea-growing region of the State, and that the general rain of June 6 was in many places the only precipitation during the entire season which wet the soil for even a brief period after temperature permitted infection of plants. Thus the fact that conditions suitable for infection did not occur until comparatively late in the season and were maintained for so brief a period reduced the amount of infection and injury from this disease to a very small amount that year.

In 1922 the onset of the disease was not as sudden or as severe. Some infected plants were found first on May 29, and thorough decay of entire root systems had occurred on June 2. Unfortunately no soil-temperature records were kept at Madison during the month of May of this year which can be compared with the records of 1923 previously cited. However, the Madison Monthly Meteorological Summary shows that the warmest weather of the month occurred May 9 to 12 during a dry period. Precipitation of over 2 inches occurred May 23 to 26, followed by six days with an average mean daily air temperature of 17.7° C., an average which is over 3° higher than of the six days after the rain of May 19, 1923, when no infection occurred. If this increase in air temperature was accompanied by a corresponding increase in soil temperature, a safe assumption since the weather record showed that they were days of almost uninterrupted sunshine, it would be just sufficient to provide a favorable temperature for infection, as determined in controlled experiments. It will, however, be necessary to accumulate many exact observations of the date at which disease appears and records of soil temperature and rainfall preceding the appearance of disease before it will be possible to predict accurately from weather records alone when the disease may be expected to appear; but it seems not unlikely that the time will come when such prediction can be made with precision.

## RELATION OF THE PARASITE TO THE HOST

### METHODS AND RESULTS OF EXPERIMENTAL INOCULATION IN THE FIELD

It will not be a matter of surprise that a fungus with a mycelium which is converted into perishable zoospores very readily and which forms oospores apparently under such restricted environmental conditions is not only limited in its ability to persist in soils, but, when present, is not always in active condition ready to invade plants. To secure such early and complete ruin of plants from inoculations with pure cultures, as often occurs in the fields, is not always an easy feat. The first difficulty which was encountered, though it appears to have been quite exceptional, deserves passing note. The first culture of this species, which was isolated, proved in the preliminary inoculation experiments to have so slight a degree of pathogenicity that the senior writer was misled and was delayed in recognizing the true cause of this disease. There is no apparent reason for the lack of pathogenicity of this isolation, and none of the seven later isolations which have been used in inoculation experiments have been thus deficient, though there appears to be a slight difference in virulence between some of them.

For greenhouse experiments inoculation of the soil by mixture of fragments of culture on corn-meal agar which have many mature oospores is the most satisfactory. Although such soil does not often give more than 50 per cent severe infection when first used, on a second planting will usually give better results, especially if care is taken to prevent drying out at any time. The use of zoospores in soil, either at the time of planting or poured on the soil when the plants have emerged, is uncertain. When plants are grown in sand, however, zoospores poured around young plants have given very thorough infection, provided great care is taken to keep the sand saturated for a time after inoculation is made.

Field inoculations have been made only at Madison, Wis., in 1923, a year so dry that it was everywhere unfavorable for the development of the disease. Inoculum was prepared in

several forms—earth previously inoculated with cultures in greenhouse experiments, cultures containing oospores planted with the seed, and zoospores applied at planting and at various times later. It seems inadvisable to present detailed results obtained thus in a single unfavorable season. Suffice it to say that some infection was obtained by all methods, but that the effectiveness of the method seemed to depend more upon the rainfall and temperature at and immediately after inoculation than upon the method. Zoospores poured upon the soil about Alaska peas breaking ground just before a rain on May 17 gave as high as 86 per cent infection, and when the soil was removed, exposing about 2 inches of the stem before the spores were distributed, every plant became infected, as evidenced by examination of the roots. Conspicuous decay following this infection of roots did not begin in any case, however, until the disease developed in plants growing on naturally infested soil. This simultaneous development of disease in plats whether infested naturally or artificially suggests that soil temperature controlled this development regardless of the time and manner of infection. All diseased plants in plats inoculated artifically grew to normal maturity and showed no conspicuous evidence of reduced vigor.

In contrast with these results of artificial inoculation were some data on the effects of natural inoculation in a similar type of soil not far removed where peas had been grown repeatedly for 7 years. Double rows of peas one rod long were planted on the infested soil, and extensions of these rows upon ground unused for peas were likewise planted with 400 peas to each rod. Unfortunately the disease had spread somewhat to the new land, so that many roots became infected, especially those of the susceptible variety, Surprize. However, the peas on the infested land showed early in the season all the symptoms of severe root rot, whereas those on the new land showed little indication of disease except at the ends of the rows adjoining diseased ground. Careful examination of plants in these plats during the season failed to reveal any other disease than that caused by Aphanomyces. The yields of some of these rows are given in Table IV.

The striking contrast between the results of natural infection and of artificial inoculation in the field plats is certainly not due to actual killing of the plants and demands explanation.

TABLE IV.—*Yields of dry peas in one rod of double row of peas upon soil heavily infested with Aphanomyces euteiches compared with yields upon adjoining ground where peas had not been grown previously. Planting was made April 28, 1923; 400 peas were planted to each rod of double row*

| Variety | Infested land | | New land | |
|---|---|---|---|---|
| | Number of plants growing | Yield | Number of plants growing | Yield |
| | | *Grams* | | *Grams* |
| Alaska | 333 | 250 | 367 | 420 |
| Do | 369 | 210 | 378 | 393 |
| Surprise | 237 | 60 | 267 | 167 |
| Do | 297 | 71 | 292 | 210 |
| Alaska | 364 | 172 | 346 | 328 |
| Do | 360 | 125 | 358 | 318 |

Very careful and frequent examinations were made of the roots in all plantings. From these examinations it was found that the number of infected roots of the plants inoculated artificially were few and restricted to the region in which the inoculum had been applied, whether above, below, or near the seed. Under the conditions obtaining during this season, even the complete destruction of the cortex of the base of the stem rarely injured a plant greatly, provided most of the roots remained free from invasion. Although in artificial inoculations trenches in which peas were planted were drenched with zoospore suspension at the time of planting, infections were few and only near the seed. No method of inoculation after the plants had started growth seems to have filled the soil very thoroughly with the parasite in such condition that it was ready to invade the plant at a large number of points at once when favorable conditions for such infection occurred. It appears from field observation described elsewhere, as well as from these experiments, that this disease can produce conspicuous injury to top growth only when plants are either infected under favorable conditions for the development of the parasite when the plants are small or when they suffer a large number of infections at a later stage of growth. In these artificial inoculations the fungus was not distributed deeply and thoroughly enough in the soil to make possible a great number of infections when the brief favorable period for infection arrived; so brief was the favorable period that inoculations of young plants made when it

arrived did not have time to give a great number of infections before dry weather checked the growth of the fungus and prevented further infections and perhaps checked the progress of the fungus through the host tissue.

## DISSEMINATION OF THE FUNGUS

The character of the fungus described in the previous pages points clearly to certain obvious methods whereby it may be distributed. Any transfer of soil from diseased fields will carry the parasite with it. In localities where soil from old fields is used to inoculate new fields with the bacteria which produce the beneficial nodules on the roots the parasite also will be carried if it is present. An excellent example of the harm which may be brought about in this way was seen in 1922 in Maryland. Soil from an old pea field in which the present crop was withering before maturity because of this root rot had been used to inoculate several neighboring fields used for peas for the first time. In every one of these fields thus inoculated individual plants scattered uniformly were dying, and others were infected, not enough in all to reduce the yield of this first crop appreciably, but enough to infest the soil so thoroughly that the failure of future crops was assured. Since transfer of soil from old fields to new may carry any of the known pea diseases which happen to be present, this practice is so often the cause of ruin to crops which are grown two or three years after the fields have thus been inoculated with disease that it appears to produce on the whole much more harm than benefit.

The flow of surface water over adjacent fields is an excellent conveyor of the fungus to new ground. The course which floods have taken across fields is sometimes indicated by areas of blighted peas. Irrigation water may be an excellent conveyor of the parasite. When the soil of an infested field is light and blown by wind, it may be a source of infestation for a large territory. In this manner a few centers of infestation are believed by R. E. Vaughan, who is familiar with the territory, to have ruined the entire area of the Truax Prairies in Eau Claire County, Wis., for pea growing. In the spring of 1922 some early plantings of peas were visited at Rochelle, Ill., when a large part of the land surface was being prepared for planting. A violent wind was drying out the surface soil so rapidly and picking up so much dust from it that the whole level country seemed covered with a fog clinging close to the ground. The distribution of spores with soil particles from a few infested fields in this locality under such conditions may account for the occasional diseased plants which are sometimes found when peas are grown for the first time in this vicinity, and the rather rapid subsequent increase of infestation.

There is a widespread opinion among growers that this disease is often introduced into new fields by the use of infected pea seed grown in diseased fields. This opinion, however, does not appear to be justified. Since the fungus does not enter the plant above the ground, it never reaches the seed and could only become attached to the seed in particles of dust from the soil. Even though this might conceivably happen, it appears to be a remote possibility, and, in any case, very few of the spores which could withstand drying would be present, so few that they would almost certainly escape detection by any experimental method that could be used and, therefore, so few that the disease which they would produce would not become conspicuous until several successive crops of peas have been grown. Although it is impossible to say that new infestation never takes place in this manner it appears to be at least a rare occurrence.

## CONTROL MEASURES

### CROP ROTATION

Although from early times the tradition of pea growing warns of failure which will attend the planting of peas on the same ground repeatedly, and English experience has developed a rotation of five or six years' duration as an insurance against disease, there are, on the other hand, so many advantages in intensive culture of the crop for the commercial purposes for which it is chiefly grown in the United States that rotation has usually been adopted only when the failure of the crop made this course necessary. A long rotation has been found to have a number of disadvantages of more or less importance. In some localities experience has abundantly shown that the second crop of peas on new land thrives better than the first, and the third may produce a better crop than either of the preceding. Peas grown for canning can often be produced more cheaply in a limited area close to the canning plant than in the much larger territory required by a long rotation. In the pea-seed-growing dis-

tricts of Montana, Idaho, and other Western States, this crop is sometimes more profitable than others, and since the suitable land area is limited the temptation to grow peas repeatedly is strong. Under the pressure of these economic reasons which favor the intensive culture of peas, many questions regarding rotation have become of vital interest to growers—how long peas may be grown before rotation is necessary, how short the rotation can be made with safety, and whether perchance new pea-growing districts may be found from which disease can be excluded or in which it will not develop.

Rotation as a control measure in combating pea diseases is generally discussed, not as a remedy for one of the many diseases of foliage and of roots, but as a control measure suited to secure relief from all of them in so far as they occur in the locality. Therefore, even though it is properly outside the limits of this paper to discuss more than the rotation that is needed to secure protection from the root rot caused by *Aphanomyces euteiches*, it will be futile to recommend a rotation which is suitable to protect from this disease alone. In the following pages this matter has been kept in mind, and, although it will be shown that a suitable rotation for peas is a local matter which must be determined with reference to the soil and climatic conditions existing there, it may be said that it appears generally true that any rotation which will control this form of rootrot will be adequate to hold other diseases sufficiently in check, at least in so far as rotation can control them.

First of all, it may be said that even when peas are grown intensively, an adequate inspection of fields can always or nearly always detect the presence of disease before it has begun to reduce yields, so that the crop can be discontinued on infested soil before losses have occurred. New soils have never been found heavily infested with the fungus unless perchance there is a badly infested field near by. The disease first appears under usual conditions in small areas, whence it spreads in successive years to the rest of the field. Usually the disease does not reach serious extent until the second year after diseased plants can be readily found. Careful inspection of fields can be made an adequate safeguard against loss wherever there is economic advantage in growing peas intensively, provided peas are not returned to land on which disease has appeared for many years.

It is very difficult to predict how soon disease will begin to appear upon any given tract of land. Experience shows enormous variation in this regard. Sometimes the third successive crop is rendered unprofitable, while neighboring fields may grow peas for 5 or 6 years successfully, and there are instances known both in Wisconsin and in irrigated districts in which peas have been grown in fields nearly every year for 10 or 12 years without a single failure. There are two possible explanations for such differences in time before the appearance of disease; either the parasite may have been absent originally from the fields which grew peas without disease for the longer period, or some soil condition may have prevented its rapid distribution and development. There is some evidence which indicates that the first condition furnishes the explanation for the late appearance of disease in some districts where peas have not been grown previously. There is a widespread belief among growers that fields in a new district will grow peas successfully much longer than fields in a district where root rot is known. That this is not always true is shown by the following instance. In the summer of 1922 a canning factory in Wisconsin far north of any locality where peas have been grown intensively was growing its third crop of peas. One field had been planted the three years in succession, four had been planted two years, and the remaining fields were on new ground. When an examination of these fields was made, about 25 per cent of the plants in the field producing its third crop were found dying with root rot, a few small groups of infected plants were found in each of the fields producing the second successive crop; while no diseased plants were discovered in any of the several other fields that were carefully searched. Although it may be true that the absence or scarcity of the fungus from some localities may explain in part the tardy appearance of root rot, it is certainly difficult to obtain adequate evidence in support of such an opinion.

On the other hand, there is abundant evidence that the disease develops much more rapidly and severely in fields upon some soil types than in neighboring fields upon other types. For instance, fields on Superior red clay in Wisconsin appear to develop root rot earlier and more severely than on any other type of soil. A minor factor that may contribute to the severity of the disease upon this type of soil may be due to the fact that, because it can not usually be worked in the spring

as early as lighter types, planting is delayed, so that the plants are younger and more readily destroyed when conditions favorable for the disease develop. This condition does not furnish an adequate explanation for the prompt appearance of the disease with intensive culture on this soil, however. Another soil type upon which the disease develops with almost equal severity, though usually at a later stage in the development of the plant, is a very sandy soil in the trucking district south of Baltimore, Md. Although it is not obvious at first that there is any common condition in these extreme soil types that causes them to be favorable for the development of the disease, nevertheless observation of conditions in these districts and in others which will be cited later has led to the conclusion that it is the retention of water in both soils for a long period after rains that furnishes the favorable environment for the fungus. The Superior red clay is rather impervious to the passage of water and when once thoroughly saturated retains surface water in hollows and in the soil structure more persistently than do lighter types of soil. This offers conditions favorable for the germination of the oospores of Aphanomyces which may be present and perhaps for the production of zoospores. On the other hand, the sandy soil referred to is underlaid with an impervious subsoil, which, in more level fields, holds standing water in the lower portion of the sand for several days after heavy rains, thus providing a similar environment for the development of the parasite.

From this it appears that soil which is naturally retentive of water or in which water is held by reason of its relation to impervious layers furnishes the most favorable condition for the development of the disease. Such a supposition is rendered very plausible by the character of the fungus and is supported by other evidence, gathered not only in humid regions but in irrigated districts as well. The best examples of the relation of soil type and method of irrigation to the development of root rot have been found in the Gallatin and Paradise Valleys in Montana and near St. Anthony, Idaho. In the Gallatin Valley in 1921 this disease was found for the first time in a small field which appeared to have been overirrigated. The condition of the roots indicated that the infestation had developed after the plants were nearly grown, and although the root systems were thoroughly rotted the yield from this field was not apparently reduced. Since this was the first observed instance of the development of the disease in this valley, and it offered an excellent opportunity to obtain evidence which would indicate the severity which the disease might attain here, arrangement was made with the canning company for whom the peas were grown to have a portion of this field replanted with peas the following year. Such a field would not have produced an average crop of peas in a nonirrigated territory. Unfortunately the senior writer was unable to revisit this valley in the following year, but the canning company reported a normal yield of peas with no conspicuous evidence of disease. When the valley was revisited in 1923, a careful search of fields reported to have been used for this crop almost continuously for 10 or 12 years yielded but a single plant which was unmistakably diseased by this fungus. So far as this valley is concerned, there is no doubt that the fungus is present and that there are abundant facilities for its rapid distribution; yet, save possibly in cases of overirrigation, it appears that it is unable to become injurious. In contrast with conditions in this valley, fields in the Pine Creek district of the Paradise Valley about 25 miles distant from the Gallatin Valley were found thoroughly infested and greatly injured by root rot both in 1921 and 1923. Peas have been grown extensively in both valleys for at least 12 years under very similar climatic conditions. Whether a difference in soil structure or difference in the practice of irrigation has produced conditions favorable to the development of the disease in the Paradise Valley is not unmistakably apparent from observation.

No soil survey has yet been made of the Paradise Valley whereby soil formation there can be compared with that in the Gallatin Valley. There are, however, conspicuous differences in topography which suggest a difference in origin of the soils and a structure more retentive of water and conducive to seepage from higher levels in some fields of the Paradise Valley.

If the water-holding capacity of soil or the retention of water in the soil is so necessary for the development of root rot, we may ask again whether the apparent immunity from disease which some Wisconsin fields have shown may not be due to soil conditions which prevent the development of the parasite. Before a final answer is given to this question a large number of fields which

are known to have enjoyed this freedom from disease must be examined. In a conspicuous instance of such immunity examined recently, the subsoil of the field was found to be sand, which afforded admirable drainage. It may be that a limited amount of land will be found outside of irrigated territory on which peas may be grown intensively for a long time without trouble from root rot. However, most soil which has drainage and texture open enough to secure immunity to disease will probably be found too dry and infertile to produce large yields from a plant with a comparatively small root system like the pea.

Since there is a large amount of valuable land now incapable of producing profitable crops of peas because of infestation with root rot, the question is often asked how long the fungus remains in the soil, and if a rotation can be devised that will make possible the utilization of this land in the future. Since there is no experimental evidence which will aid in answering these questions, it is necessary to seek the experience of growers who have abandoned land for pea growing because of disease for varying periods of years. Experience of this kind is always open to question because one can not be sure that this root rot and not some other disease was the cause of the crop failure, both before and after the period in which the land was used for other crops. Notwithstanding such doubts, there appear to be two well-attested instances in Wisconsin in which fields failed to produce healthy peas after being used six years for other crops following a complete failure from rootrot. The writer has seen two fields of peas ruined by root rot after an interval of four years following a failure and another field in the same locality producing healthy peas nine years after a failure, presumably from this disease. In contrast with this Wisconsin experience, healthy fields of peas have been found in sandy soil in Delaware where peas failed four years earlier from disease which may have been root rot. From these instances and other experiences which might be cited it seems that in the heavier soils the parasite may persist for at least six years after it has once become abundant. In light soils its period of survival may be shorter. Probably no other fungus parasite of peas survives so long as this, and its duration in any locality can best be determined by repeated experimental plantings on infested ground during a series of years.

### RESISTANT VARIETIES

Observation of the behavior of varieties of peas grown on diseased soil in several places has given rise to the opinion, more or less current among growers, that some varieties of peas show resistance and will produce a better crop than others. Inasmuch as Wilber Brotherton has been studying resistance under field conditions on an extensive scale and will report results in the near future, no attempt will be made here to consider the merits of commercial varieties. An attempt has been made to supplement Brotherton's studies by seeking to devise laboratory methods whereby disease resistance can be tested more rapidly than in field trials, and also to learn in so far as possible the nature of the resistance which varieties may possess.

The attempt to test varieties in the greenhouse under controlled conditions has not yet developed a method that is satisfactory. In the greenhouse in winter there is an apparent obliteration or reduction of differences in behavior compared with that observed in the field not unlike that which occurred in an earlier study (*8*, p. 471) of resistance of peas to a species of Fusarium. This work is still in progress and may be reported more fully later.

It may be said here, however, that no variety of peas, whether of the garden or of the field varieties, has been found immune to the fungus. The hardiest variety of field pea which produces a crop on soil so infested that few plants of garden varieties survive to maturity suffers almost if not quite as great a loss of cortex from its roots as its more susceptible neighbors. In spite of this loss it maintains growth and matures seed. This difference in behavior, termed resistance, seems to be due to several characters which resistant plants possess to a greater degree than those which perish. There is some evidence indicating that the fungus traverses the cortex of some varieties much more slowly than that of others, thus destroying roots less rapidly. Finally, when cortex is destroyed the remaining vascular cylinder of some plants seems to be able to exclude bacteria and fungi in the soil and to function quite efficiently thus denuded of absorbing tissue.

Whatever the importance of these several factors in resistance mentioned above, it will be seen at once that the growth of resistant peas

permits the infestation of the soil with the fungus to proceed as rapidly as when the most susceptible peas are grown. With intensive culture these resistant peas will be subjected to increasingly severe infection from year to year. Resistant varieties, however, may afford a very satisfactory escape from root rot where it is not extremely severe. But it remains to be determined experimentally whether any resistance has yet been found in varieties of commercial utility which will continue to produce profitable crops with intensive culture on soil in which this disease reaches its greatest severity.

## SUMMARY

1. Of the several root-rot diseases of peas occurring in the United States which have been distinguished and studied during the past five years, the disease caused by the fungus described in this paper appears to be the most important. It occurs in nearly all of the important pea-growing districts with a varying severity which depends largely upon the degree to which intensive culture of peas permits the accumulation of the fungus in the soil and upon the conditions of soil temperature and moisture favoring early infection and rapid decay of the invaded roots. No other crop than peas has been found subject to disease from this fungus.

2. The effect of this disease upon the appearance of the plant in the field and upon the yield of the crop varies with the stage of development of the plant at which infection takes place and upon the number of infections. If the root system is invaded extensively when only three or four nodes have been formed, the plant may wilt and die suddenly. Later invasions may result in dwarfing of growth with drying out of foliage from the ground upward and in unproductivity. The disease can hardly be distinguished by the appearance of the top of the plant, but it can usually be identified readily when plants are pulled from the ground by the behavior of the root, which instead of breaking near the planted seed, pulls out as a fibrous string consisting of the vascular cylinder of the root freed from the decayed cortex.

3. The fungus enters only the cortex of the roots and base of the stem, where it produces a softening and rapid decay of the tissue, leaving the vascular cylinder exposed to decay by other organisms. In most varieties of garden peas the smaller roots thus denuded of cortex die immediately. A large number of oospores are formed by the fungus in the invaded cortex, and it appears to be from these spores, which increase in the soil from year to year with intensive culture of the crop, that infection originates each season.

4. The fungus can be isolated in pure culture only with considerable difficulty, both because the period during which the mycelium is growing actively in the tissue is brief and because it is so closely associated with bacteria and other fungi that the separation of the parasite from its associates is not readily accomplished. However, 12 cultures from different localities have been obtained for comparison and study.

5. Within the host tissues or on a suitable solid substratum the mycelium soon gives rise to resistant thick-walled bodies, the oospores which result from the development of oogonia following their fertilization by antheridia, of which from one to five are associated with each female cell. Depending on the presence or absence of food materials, the oospores germinate either directly by the production of one to several vegetative hyphae, or indirectly by the proliferation of a single germ hypha of limited growth, within which, as well as within the oospore wall, the protoplasm is divided into portions 13 to 18 in number, which are promptly discharged in the manner characteristic of the genus.

6. Asexual reproduction resulting in the formation of great numbers of motile zoospores ensues whenever actively growing mycelium is provided with suitable conditions. Young thalli may become almost entirely involved in sporogenesis, the individual sporangia being represented by portions of mycelium delimited by septa and often including a moderate number of well-developed branches. These sporangial units discharge their zoospores by one or several elements, the distal portions of which are considerably constricted.

7. The fungus shows much similarity to two aquatic congeners possessing smooth oogonia, *Aphanomyces laevis* and *A. helicoides*, differing especially, however, from the former in having a greatly thickened oogonial envelope with characteristically sinuous internal contour, and from the latter in its antheridial branches not exhibiting any well-defined spiral habit. It is described as a new species, *Aphanomyces euteiches* Drechsler.

8. Inoculation of pea plants with pure cultures under conditions of controlled soil temperature and moisture show that infection of peas may take place at temperatures between 10° and 30° C., but that optimum

temperature for development of the disease is approximately between 15° and 30°. Differences in soil moisture gave little difference in infection under the conditions of these experiments, whereas observation in the field seems to show that the disease is more severe on soil with high moisture-holding capacity or on soil in which water is held by impervious subsoil or by subirrigation.

9. The fungus appears to occur widely in cultivated soils and may be conveyed from infested fields to others by any agency that carries soil. There is no evidence indicating that it is carried with seed.

10. The disease can be prevented and controlled most effectively by crop rotation. The length of rotation required appears to vary greatly with local conditions. On certain irrigated soils which appear to have such low moisture-holding capacity that they provide unfavorable conditions for the development of the fungus, the disease has not appeared or has not become destructive even after peas have been grown nearly every year for 10 years. The disease has become destructive on similar soil when it is subirrigated. On some soils in humid territory the third successive crop of peas is often badly damaged by this root rot, and a comparatively long rotation appears to be necessary to prevent it from accumulating in the soil. In some soils which have become heavily infested the fungus appears to have persisted in sufficient amount to produce conspicuous injury to peas after a six-year rotation.

11. Although all varieties of field and garden peas are subject to this disease, there is considerable difference in the amount of injury which they incur from it, especially in situations where the disease does not develop great severity. Study of the nature and commercial value of this resistance is in progress.

## LITERATURE CITED


(1) BARY, A. DE.
1860. EINIGE NEUE SAPROLEGNIEEN. Jahrb. Wiss. Bot. [Pringsheim] 2: 169–192, illus.

(2) CLINTON, G. P.
1920. NEW OR UNUSUAL PLANT INJURIES AND DISEASES FOUND IN CONNECTICUT, 1916–1919. Conn. Agr. Exp. Sta. Bul. 22, p. 397–482, illus.

(3) COKER, W. C.
1923. THE SAPROLEGNIACEAE, WITH NOTES ON OTHER WATER MOLDS. 201 p., illus. Chapel Hill, N. C.

(4) HARTLEY, C.
1921. DAMPING-OFF IN FOREST NURSERIES. U. S. Dept. Agr. Bul. 934, 99 p., illus.

(5) HUMPHREY, J. E.
1893. THE SAPROLEGIACEAE OF THE UNITED STATES WITH NOTES ON OTHER SPECIES. Trans. Amer. Phil. Soc. (n. s.) 17: 63–148, illus.

(6) JONES, F. R.
1920. PYTHIUM AS A CAUSAL FACTOR IN "PEA BLIGHT." Phytopathology 10: 67.

(7) ———
1923. ROOT-ROT OF PEAS IN THE UNITED STATES. Rpt. Internat. Conf. Phytopath. & Econ. Ent. Holland 1923: 203–204.

(8) ———
1923. STEM AND ROOTROT OF PEAS IN THE UNITED STATES, CAUSED BY A SPECIES OF FUSARIUM. Jour. Agr. Research 26: 459–476, illus.

(9) ———
1925. A MYCORRHIZAL FUNGUS IN THE ROOTS OF LEGUMES AND SOME OTHER PLANTS. Jour. Agr. Research (1924) 29: 459–470, illus.

(10) KASANOWSKY, V.
1911. APHANOMYCES LAEVIS DE BARY. I. ENTWICKLUNG DER SEXUAL-ORGANE UND BEFRUCHTUNG. Ber. Deut. Bot. Gesell. 29: 210–228, illus.

(11) MINDEN, M. VON.
1912. PILZE I. PHYCOMYCETES. APHANOMYCES. Kryptogamenflora der Mark Brandenburg 5: 555–562, illus.

(12) NĚMEC, B.
1913. ZUR KENNTNIS DER NIEDEREN PILZE. VI. EINE NEUE SAPROLEGNIAZEE. Bul. Internat. Cl. Sci. Math., Nat., Med., Česká Akad. Cis. Frant. Jos. 18: 32–43, illus.

(13) PETERS, L.
1907. WURZELBRAND DER ZUCKERRÜBEN. Umschau 11: 85–87, illus.

(14) ———
1911. ÜBER DIE ERREGER DES WURZELBRANDES. Arb. K. Biol. Anst. Land-u. Forstw. 8: 211–259, illus.

(15) ROTHERT, W.
1903. DIE SPORENENTWICKELUNG BEI APHANOMYCES. Flora 92: 293–301, illus.

(16) SOROKINE, N.
1876. QUELQUES MOTS SUR LE DÉVELOPPEMENT DE L'APHANOMYCES STELLATUS. Ann. Sci. Nat. Bot. (VI) 3: 46–52, illus.

(17) WEATHERWAX, P.
1914. APHANOMYCES PHYCOPHILUS DE BARY. Proc. Ind. Acad. Sci. 1913: 109–111, illus.

(18) WILLE, N.
1899. OM NOBLE VANDSOPPE. Skr. Vidensk. i Christiania. I. Math.-Naturvidensk. Kl. 1899, no. 3, 14 p., illus.

(19) WITTMACK, L.
1892. PYTHIUM SADEBECKIANUM ALS URSACHE EINER KRANKHEIT DER ERBSEN. Mitt. Ver. Förderung Moorkult. 10: 83. [Original not seen. Resumé by E. J. Butler in Mem. Dept. Agr. India Bot. Ser. 1 (5): 105.]


13952—25†——3

# DIRECT INOCULATION OF CONIFEROUS STEMS WITH DAMPING-OFF FUNGI [1]

By ANNIE RATHBUN-GRAVATT

*Junior Pathologist, Office of Forest Pathology, United States Department of Agriculture*

## INTRODUCTION

Hartley's (*2*, *3*) [2] and Spaulding's (*8*) summaries of the important literature on the damping-off of conifers have brought out the fact that aerial stem infections are less frequent than subterranean infections. Most of these published reports have been based upon field observations or upon experiments in which inoculum was added to the soil at the time the seeds were sown, instead of being applied directly to the seedlings. These methods, while giving the combined loss from the various types of damping-off, (germination loss, root damping-off, stem damping-off, etc.), are not entirely satisfactory for showing just what part of this loss each type causes. Results secured from the direct inoculation of coniferous taproots have been published previously (*6*). This paper will present the results from the direct inoculation of coniferous stems.

## METHODS OF INOCULATION

The seedlings for most of the experiments were grown in autoclaved sand in 2-inch pots in the greenhouse of the United States Department of Agriculture at Washington, D. C. They were watered only with boiled water. The pots were kept from the time of seeding until the time of inoculation in glass-walled cupboards previously disinfected with mercuric chloride. At the time of inoculation the pots were taken from the cupboard and all but 10 seedlings were removed from each pot. The seedlings were inoculated while the seed-coats were still clinging to the cotyledons of most of them. The seedlings grown in this way, though normally green, were taller than seedlings of the same age usually are, the light intensity in the glass cupboards being apparently insufficient for normal growth.

The original plan was to inoculate each seedling at the soil surface because usually in seedbeds aerial infections occur at the soil surface rather than above. Almost immediately, however, this method was discarded in favor of inoculation platforms (fig. 1), since it was found very impractical to test the fungi by inoculation at the soil surface without the fungi's growing from one seedling to another through the soil. The method adopted is believed to give a fair indication of the results which would be obtained at the soil surface. The platforms consisted of equilateral triangles with sides three-eighths of an inch long cut from library cards. A toothpick was inserted through a hole in the center of each triangle. An inoculation platform was placed beside each seedling, and upon it in contact with the stem was placed the inoculum. Each toothpick was pushed into the soil until the triangular platform was about three-eighths of an inch above the soil surface.

Inoculum for each seedling consisted of either a 4 by 4 by 2 mm. cube of corn-meal agar, or rice mush about the size of a swollen rice grain, plus the mycelium and any spores growing upon it. In case both kinds of inoculum were used in the same experiments one kind was used on 5 seedlings, and the other kind on the 5 seedlings on the other side of the same pot. Each control pot was divided in the same manner, each half being treated with one kind of sterile medium. In each experiment there were usually 150 control seedlings, 75 for each medium when two kinds were used. At least 10 seedlings were inoculated with each fungous line grown on each medium.

Individual damp chambers were made for each pot, consisting of truncated sheet celluloid cones 5 inches high and 2½ inches in diameter at the base. Moisture was furnished by

[1] Received for publication, June 5, 1924—issued, April, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 339.

plugging the top of each cone with cotton wet in boiled water. In case the sand itself became too dry the pots were set in pans of boiled water. As soon as damp-chamber conditions were no longer desired (usually 2 days after inoculation) the cotton was removed from the top of the cone. Before use, the cones were disinfected with formalin or mercuric chloride solution.

After inoculation, the pots were placed on stands whose legs were set in pans of water in order to exclude slugs and ants. These methods of inoculation, together with others, have been described and illustrated in a previous paper (*4*). Each experiment was allowed to run four days. A daily count was made of the damped-off seedlings and those found diseased were removed. Only those seedlings which began to damp-off at the point of inoculation were considered (fig. 1).

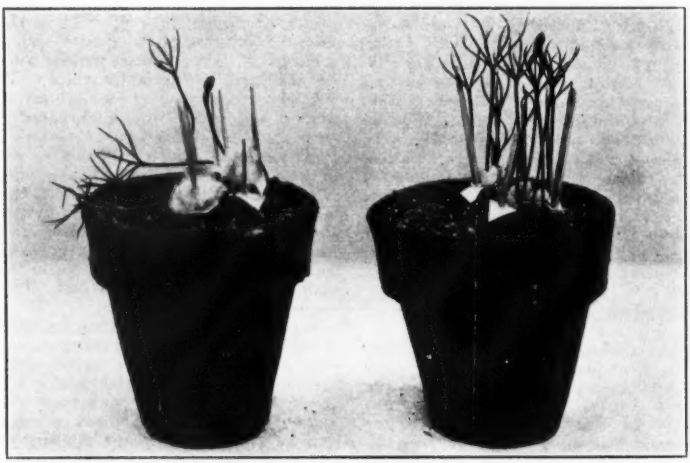


FIG. 1.—Pot at left shows damping-off caused by *Pythium debaryanum*. Pot at right shows seedlings treated at same time with sterile rice grains

It was undesirable to use the above method for such exotic fungi as *Phytophthora fagi* and *Pythiacystis citrophthora*. Therefore seedlings grown in plugged test tubes were inoculated with these two fungi. Sand was placed in plugged test tubes and the whole sterilized in the autoclave. Then the seeds were planted and watered with sterile water. In each tube were two or three seedlings. The inoculum which consisted of either corn meal or rice as above was placed on the surface of the sand in contact with the seedlings. The plug remained in the tube during the whole experiment, thus making a damp chamber. At the end of each experiment each tube was again autoclaved to prevent the escape of the fungi.

The results secured by the above methods of inoculation involving heavy inoculum for each seedling, seedlings grown on sterilized sand, boiled water, individual disinfected damp chambers, etc., do not constitute an entirely reliable index to the manner in which these fungi would act in ordinary seedbeds. They do, however, give some idea of the relative virulence and importance of the different genera of fungi and show which can attack coniferous stems under favorable conditions. The fungi which were only weakly parasitic in these tests would probably be entirely harmless under ordinary nursery conditions.

## THE TESTED FUNGI

Over 100 different fungous lines were tested by the methods described above. By the term "line" as used in this paper is meant a fungous culture arising from one isolation. Although the cultures of Pythium, Corticium, and some others were isolated from tissue or soil plantings and were not entirely single lines, all except No. 230, maintained constant behavior through a sufficient number of generations to give fair assurance of their purity. Results obtained with many of the same lines have been published elsewhere (*2*, *3*, *5*, *6*). In Table I are given

the line number, the host, the locality, and the person to whom each line originally belonged.

### Technical Description of Phytophthora Sp.

A brief description is given below of the Phytophthora isolated from pine. This Phytophthora grew well on cornmeal agar, sterilized beans, peas, beets, and turnips.

Hyphae approximately 2.5 to 6.5 μ in diameter. Both aerial and submerged, flexuose and noduled with characteristic swellings at end of branches.

Sporangia approximately 19 to 40 μ by 28 to 86 μ. Not produced on ordinary media. Only produced when the hyphae alone, or the hyphae plus the substratum are placed in running water and kept at temperature of about 12° C. Germination by a tube or by formation of zoospores. Resting spherical zoospores approximately 7 to 9 μ.

TABLE I.—*Source and identity of the tested fungi* [a]

| Name | Line No. | Source | | |
|---|---|---|---|---|
| | | Host | Locality | Contributed by— |
| *Botrytis cinerea* Pers. | 470 | *Pseudotsuga taxifolia* seedling. | Utah | C. Hartley. |
| | 920 | *Ceanothus americanus* | New York [b] | H. H. Whetzel. |
| | 921 | *Ribes rubrum* | do | Do. |
| | 924 | *Rosa* sp. | do | Do. |
| | 925 | *Arachis hypogaea* | do | Do. |
| | 944 | *Hibiscus sabdariffa* | California | W. T. Horne. |
| *Botrytis* spp. (small sclerotial types). | 922 | *Arisaem atriphyllum* | New York [b] | H. H. Whetzel. |
| | 923 | *Hydrastis canadensis* | do | Do. |
| *Cephalothecium roseum* Cda. | 288 | *Ilex* nursery stock | District of Columbia | C. Hartley. |
| *Corticium vagum* B. and C. (*Rhizoctonia solani* Kühn). | 50 | *Pinus banksiana* seedling | Nebraska | Do. |
| | 147 | *Picea engelmanni* seedling | Districtof Columbia | Do. |
| | 183 | *Phaseolus vulgaris* | New York | M. F. Barrus. |
| | 186 | *Solanum tuberosum* | Ohio | Mrs. C. R. Tillotson |
| | 187 | do | New York | Do. |
| | 189 | *Beta vulgaris* | Michigan | Mrs. L. J. Weld. |
| | 205 | *Pseudotsuga taxifolia* | Colorado | Mrs. H. E. Watkins. |
| | 213 | *Beta vulgaris* seedling | District of Columbia | H. A. Edson. |
| | 230 | *Elaeagnus* sp. | Garden City, Kans. | C. Hartley. |
| | 233 | *Elaeagnus sp.* (duplicate of No. 230). | do | Do. |
| | 240 | *Pinus ponderosa* seedling | do | T. C. Merrill. |
| | 329 | Reisolation of No. 147 from *Pinus ponderosa* seedling. | | R. G. Pierce. |
| | 330 | Reisolation of No. 230 from *Pinus banksiana* seedling. | | Do. |
| | 331 | Reisolation of No. 213 from *Pinus banksiana* seedling. | | Do. |
| | 332 | Reisolation of No. 147 from *Pinus ponderosa.* | | Do. |
| | 333 | Reisolation of No. 147 from *Pinus ponderosa* seedling. | | Do. |
| | 340 | Reisolation of No. 213 from *Pinus banksiana* seedling. | | Do. |
| | 341 | Reisolation of No. 230 from *Pinus banksiana* seedling. | | Do. |
| | 343 | Reisolation of No. 147 from *Pinus banksiana* seedling. | | Do. |
| | 361 | *Pinus resinosa* seedling | Cass Lake, Minn. | Do. |
| | 362 | do | do | Do. |
| | 363 | do | do | Do. |
| | 365 | do | do | Do. |
| | 380 | do | do | Do. |
| | 381 | *Pinus strobus* seedling | do | Do. |
| | 552 | *Picea engelmanni* seedling | California | C. Hartley. |

[a] The writer wishes to express her thanks to Miss Helen Johann, of the Office of Cereal Investigations, U. S. Department of Agriculture, for the identification of Atanasoff's Fusarium cultures; to Dr. C. D. Sherbakoff, of the Tennessee Station, for the identification of several other Fusarium cultures; and to Dr. Carl Hartley, of the Office of Forest Pathology, U. S. Department of Agriculture, for his kind assistance and valuable suggestions.

[b] Locality not positive.

[c] Identified by C. D. Sherbakoff.

TABLE I.—*Source and identity of the tested fungi*—Continued

| Name | Line No. | Source | | |
|---|---|---|---|---|
| | | Host | Locality | Contributed by— |
| *Corticium vagum* B. and C. (*Rhizoctonia solani* Kühn). | 721 | *Pinus resinosa* seedling | East Tawas, Mich. | G. G. Hahn. |
| | 723 | do | do | Do. |
| | 724 | do | do | Do. |
| | 746 | do | do | Do. |
| | 747 | *Pinus resinosa* seedling (duplicate of No. 746). | do | Do. |
| | 761 | *Pinus resinosa* seedling | do | Do. |
| *Fusarium* spp. | 416 | *Pinus banksiana* seedling | District of Columbia | R. G. Pierce. |
| | 446 | *Picea engelmanni* seedling | do | Do. |
| *Fusarium acuminatum* E. and E. | 283 | *Pinus ponderosa* seedling | do | T. C. Merrill. |
| | 508 | *Pseudotsuga taxifolia* seedling. | Utah | C. Hartley. |
| | 932 | *Ipomoea batatas* | District of Columbia | L. L. Harter. |
| *Fusarium arthrosporioides* Sherb. | 908 | | | D. Atanasoff. |
| *Fusarium avenaceum* (Fr.) Sacc. | 911 | | | Do. |
| *Fusarium coeruleum* (Lib.) Sacc. | 934 | *Solanum tuberosum* | New York[b] | H. A. Edson. |
| *Fusarium culmorum* (W. G. Sm.) Sacc. group. | [c]557 | *Picea engelmanni* seedling | California | C. Hartley. |
| | 917 | | | D. Atanasoff. |
| | 929 | *Ipomoea batatas* | Indiana | L. L. Harter. |
| *Fusarium discolor* Ap. & Wr. section. | [c]367 | *Pinus resinosa* seedling | Minnesota | R. G. Pierce. |
| | [c]518 | Soil | | C. Hartley. |
| | 935 | | Germany | H. A. Edson. |
| *Fusarium discolor sulphureum* (Schlecht) Ap. & Wr. | 936 | | do | Do. |
| *Fusarium elegans* group. | [c]370 | *Pinus resinosa* seedling | Minnesota | R. G. Pierce. |
| | [c]736 | do | Michigan | G. G. Hahn. |
| | [c]779 | do | do | Do. |
| | 904 | | | D. Atanasoff. |
| | 909 | | | Do. |
| | 912 | | | Do. |
| | 913 | | | Do. |
| | 915 | | | Do. |
| *Fusarium eumartii* Carp. | 937 | *Solanum tuberosum* | Pennsylvania | H. A. Edson. |
| *Fusarium hyperoxysporum* Wr. | 930 | *Ipomoea batatas* | District of Columbia | L. L. Harter. |
| *Fusarium martiella* section. | [c]268 | *Pinus ponderosa* seedling | Kansas | T. C. Merrill. |
| *Fusarium martii* Ap. & Wr. | 914 | | | D. Atanasoff. |
| *Fusarium moniliforme* Sheld. section. | 239 | *Pinus ponderosa* seedling | Garden City, Kans. | T. C. Merrill. |
| | 247 | do | do | Do. |
| | 249 | do | do | Do. |
| | 251 | do | do | Do. |
| | 260 | do | do | Do. |
| | 265 | *Pinus banksiana* seedling | do | Do. |
| | 266 | *Pinus ponderosa* seedling | do | Do. |
| | 273 | do | do | Do. |
| | [c]308 | Soil | do | R. G. Pierce. |
| | 910 | | | D. Atanasoff. |
| | [c]947 | *Zea mays* roots | Louisiana | G. N. Hoffer. |
| | [c]948 | *Zea mays* plant | Georgia | Do. |
| | [c]949 | *Zea mays* stalk | Oklahoma | Do. |
| *Fusarium orthoceras*? Ap. & Wr. | [c]357 | *Pinus resinosa* seedling | Minnesota | R. G. Pierce. |
| *Fusarium oxysporum* Schlecht. | 938 | *Solanum tuberosum* | | H. A. Edson. |
| *Fusarium radicicola* Wr. | 939 | do | Colorado | Do. |
| *Fusarium* sp. between *roseum* and *discolor* groups. | [b]946 | *Zea mays* stalks | Indiana | G. N. Hoffer. |
| *Fusarium solani* (Mart.) Ap. & Wr. group. | 202 | *Pinus ponderosa* seedling | Nebraska | C. Hartley. |
| | 905 | | | D. Atanasoff. |
| | 940 | | | H. A. Edson. |
| | 906 | | | D. Atanasoff. |
| *Fusarium sporotrichioides* Sherb. | 941 | *Solanum tuberosum* | Idaho | H. A. Edson. |
| *Fusarium trichothecioides* Wr. | 928 | *Ipomoea batatas* | District of Columbia | L. L. Harter. |
| *Fusarium vasinfectum* Atk. | 942 | *Gossypium* | | H. A. Edson. |
| *Fusarium ventricosum* Ap. & Wr. | 299 | *Pinus ponderosa* seedling | | C. Hartley. |

[b] Locality not positive. [c] Identified by C. D. Sherbakoff.

TABLE I.—*Source and identity of the tested fungi*—Continued

| Name | Line No. | Source | | |
|---|---|---|---|---|
| | | Host | Locality | Contributed by— |
| *Gibberella saubinetii* (Mont.) Sacc. | 916 | | | D. Atanasoff. |
| | 933 | *Ipomoea batatas* | Indiana | L. L. Harter. |
| *Mucor racemosus* Fres. | 927 | | | Do. |
| *Nectria ipomoeae* Hals. | 931 | *Ipomoea batatas* | New Jersey | Do. |
| *Pestalozzia funerea*? Desm. | 968 | *Pinus palustris* leaves | Florida | M. W. Taylor and G. G. Hedgcock. |
| *Phomopsis juniperovora* Hahn. | 860 | *Juniperus virginiana* | East Tawas, Mich. | G. G. Hahn. |
| | 864 | do | do | Do. |
| | 865 | do | do | Do. |
| | 867 | do | do | Do. |
| Unidentified Phycomycete. | 543 | *Olea europaea* | California | C. Hartley. |
| *Pythiacystis citrophthora* Sm. and Sm. | 943 | | | J. T. Barrett. |
| *Pythium artotrogus* (Mont.) De Bary. | 778 | *Pinus resinosa* seedlings | Michigan | G. G. Hahn. |
| | 821 | *Pinus banksiana* seedlings | District of Columbia | Do. |
| | 833 | do | do | Do. |
| *Pythium debaryanum* Hesse. | 131 | *Solanum tuberosum* | California | Mrs. C. R. Tillotson. |
| | 258 | *Pinus ponderosa* seedling | Kansas | T. C. Merrill. |
| | 295 | Reisolation of No. 131 from *Beta vulgaris* seeding. | | H. A. Edson. |
| | 296 | *Beta vulgaris* seedling | Wisconsin | Do. |
| | 338 | Reisolation of No. 295 from *Pinus ponderosa* seedling. | | R. G. Pierce. |
| | 408 | Reisolation of No. 338 from *Pinus banksiana*. | | Do. |
| | 529 | *Trigonella foenum graceum* seedling. | Sonoma County, Calif. | C. Hartley. |
| | 550 | *Picea sitchensis* seedling | Berkeley, Calif. | Do. |
| | 743 | *Pinus resinosa* seedling | Michigan | G. G. Hahn. |
| | 767 | do | do | Do. |
| | 810 | *Solanum tuberosum* | California | L. A. Hawkins. |
| *Phytophthora cactorum* (C. & L.) Sebrath. | 901 | | | L. O. Kunkel. |
| *Phytophthora fagi* Hart. | 967 | | | Joha. Westerdijk. |
| *Phytophthora* sp. | 358 | *Pinus resinosa* seedling | Cass Lake, Minn. | R. G. Pierce. |
| | 372 | do | do | Do. |
| | 843 | Reisolation of No. 372 from *Pinus ponderosa* seedling. | | G. G. Hahn. |
| *Rheosporangium aphanidermatus* Edson. | 351 | *Beta vulgaris* seedling | Wisconsin | H. A. Edson. |
| | 430 | Reisolation of No. 351 from *Pinus banksiana* seedling. | | R. G. Pierce. |
| *Rhizoctonia potomacensis* Wr. | 881 | *Lycopersicon esculentum* | District of Columbia | H. A. Edson. |
| *Thielavia basicola* (B. & B.) Zopf. | 428 | *Nicotinia* | Wisconsin | J. Johnson. |
| *Verticillium* sp. | [c]399 | *Litchi chinensis* | California | R. G. Pierce. |

[c] Identified by C. D. Sherbakoff.

Oogonia approximately 20 to 38 $\mu$. Oospores approximately 14 to 31 $\mu$. Oogonia brownish, smooth, practically spherical. Oospores smooth, spherical, lying free in oogonia. Walls about 2.6 $\mu$ thick. Oogonia apparently grow up through laterial or basal antheridia. Further taxonomic study is necessary.

## METHOD OF EVALUATING RESULTS

The next to the last column in Tables II, IV, V, and VI shows the average per cent of the seedlings killed (direct average of the preceding three or four columns). It is obvious that this average does not absolutely represent the amount of damage which each fungus can cause, because it is based upon a direct comparison of the different fungi, just as if they had all been tested in the same experiment. As a matter of fact, some of the fungi were tested in one experiment, and others in different experiments in which conditions may have been more or less favorable to damping-off. Three "standard" fungi—one line of *Pythium debaryanum*, one of *Corticium vagum*, and one of *Fusarium moniliforme*—were used in all experiments for the sake of checking the homogeneity of the results. The fact that these fungi were decidedly more virulent in some

experiments than in others shows that the conditions of those experiments were more favorable than in others for damping-off. In order to make the results of the different experiments directly comparable the data were reworked. The mortality percentage for each fungus in each experiment was corrected, in much the same way that agronomists correct plot yields for field heterogeneity.[3] For example, if the average mortality per cent for the three standard lines in experiment B was less than the average of the same lines for all experiments, the mortality percentage for each fungus was proportionally raised. If the average mortality for them was more than it was for all experiments each mortality per cent was lowered. The corrected percentages were averaged and constitute the last columns in the tables. The averages in the next to the last column of the tables are open to suspicion because the percentages on which they were based are uncorrected for the varying conditions of the experiments. The corrected averages in the last column are under suspicion because of (1) the small number of standard lines on which corrections are based; (2) the probability that the respective fungi are differently affected by changes in conditions, and (3) the somewhat questionable character of the assumption on which the method of correction is based. It is thus probable that the corrected percentages are corrected too much and that the true mortality percentages lie somewhere between the corrected and uncorrected values. The fact that the agreement between the averages of the corrected and the uncorrected percentages is reasonably good, and that the relative virulence of the fungi seems approximately the same regardless of the method of averaging, seems to indicate that a direct comparison of the different experiments introduced no serious errors. An average of the uncorrected and corrected averages would probably be a fair index to the relative virulence of the fungi tested in these experiments.

TABLE II.—*Damping-off of coniferous stems caused by Pythium debaryanum and other Phycomycetes*

| Tested fungi | Number of lines tested | | | Number of seedlings inoculated | | | | Per cent of seedlings killed | | | | | Average of corrected percentages |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | On Pinus resinosa | On Pinus banksiana | On Picea englmanni | Pinus resinosa | | Pinus banksiana | Picea engelmanni | Pinus resinosa | | Pinus banksiana | Picea engelmanni | Average | |
| | | | | Agar inoculum | Rice inoculum | | | Agar inoculum | Rice inoculum | | | | |
| Pythium debaryanum, all lines | 11 | 9 | 9 | 330 | 460 | 160 | 230 | 57 | 90 | 98 | 97 | 85 | 83 |
| Line No. 131 and its reisolations | 4 | 3 | 3 | 80 | 120 | 50 | 80 | 62 | 91 | 100 | 96 | 87 | 83 |
| Lines isolated from coniferous hosts | 6 | 4 | 4 | 120 | 180 | 70 | 100 | 55 | 86 | 100 | 98 | 85 | 84 |
| Lines isolated from other hosts | 5 | 5 | 5 | 210 | 280 | 90 | 130 | 58 | 92 | 97 | 94 | 85 | 83 |
| Rhoesporangium aphanidermatus | 2 | 2 | ---- | 40 | 40 | 40 | ---- | 58 | 83 | 95 | ---- | 79 | 72 |
| Phytophthora sp | 3 | 3 | ---- | 70 | 70 | 40 | ---- | 14 | 34 | 25 | ---- | 24 | 27 |
| Phytophthora cactorum | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 0 | 15 | ---- | ---- | 8 | 13 |
| Pythium artotrogus | 3 | 1 | ---- | 60 | 60 | 20 | ---- | 0 | 2 | 0 | ---- | 1— | 1 |
| Unidentified Phycomycete | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 0 | 0 | ---- | ---- | 0 | 0 |
| Mucor racemosus | 1 | ---- | ---- | 10 | 10 | ---- | ---- | 0 | 0 | ---- | ---- | 0 | 0 |
| Controls | ---- | ---- | ---- | 975 | 1, 225 | 450 | 300 | 0. 1 | 2. 5 | 0. 7 | 0. 3 | 1— | ---- |

[3] Obviously the percentages could not be corrected in exactly the same way however. For instance, if in all the experiments the standard lines caused an average loss of 60 per cent, but in experiment B only 40 per cent, a proportional correction of each mortality percentage in experiment B would raise a fungus which had caused 80 per cent damping-off to 120 per cent. Even in cases where there is no such obvious absurdity resulting from correction, the inevitable cramping of the percentage scale near 0 and 100 would necessitate some other method of applying corrections. The percentages on which the averages in the last columns of Tables II, IV, V, and VI were based, were corrected by the following method suggested by Dr. Sewall Wright.

It is evident that in experiment B of the above hypothetical case a mortality of 40 per cent caused by a particular fungus should be corrected to 60 per cent. In the normal frequency curve between the 40th and 60th percentile the abscissal distance on the base line is $0.5068\sigma$. A 20 per cent loss caused by another fungus in the same experiment was corrected as follows:

By reference to a normal probability integral table (*1, Table IV*) it was found that an abscissal distance of $0.5068\sigma$ on the base line to the right of the 20th percentile had over it 17 per cent of the area of the normal probability curve. The 20 per cent mortality was therefore raised to 37 per cent. The same procedure raised 10 per cent to 20 per cent, 50 per cent to 69 per cent, and 90 per cent to 96 per cent. The basis of the method is the assumption that the conditions which affect a given lot of seedlings infected with a given fungus have a certain standard deviation which is the same for all lots.

## RESULTS OF THE PRESENT INVESTIGATION

### Pythium Debaryanum

The results secured by inoculating seedlings of *Pinus resinosa* Ait., *P. banksiana* Lamb., and *Picea engelmanni* Engelm. with *Pythium debaryanum* by the platform method are given in Table II. *P. debaryanum* caused some damping-off in all but three inoculated units, all three with the same weak line (No. 743). Regardless of its substratum, *P. debaryanum* caused more than half of the seedlings to damp-off. In the case of *Pinus resinosa* both rice mush and corn-meal agar inoculum were tested. In more than three-fourths of the tests the fungi when grown on rice mush caused more damping-off than in the same tests when grown on corn-meal agar. The latter were not more destructive in a single case. Since the mycelial development was much more luxuriant on the mush, it seems that virulence may be associated with vigor of growth. Figure 2 shows this graphically. The lines isolated from coniferous hosts were no more virulent than those isolated from other hosts. On the whole, the *P. debaryanum* lines were more destructive than any of the other fungi, with the possible exception of *Botrytis cinerea*. The control seedlings damped-off very little.

An attempt was made to get some idea of the relative susceptibility of the various hosts to *Pythium debaryanum* by comparing the percentages damped-off by the eight lines tested on all three hosts with rice mush as a substratum. The figures secured in this way vary slightly from those given in Table II, where all lines were included, but for practical purposes they are the same. For *Pinus resinosa* the damping-off was 93 per cent; for *P. banksiana*, 98 per cent; and for *Picea engelmanni*, 96 per cent. Thus it seems that all three host species are highly and practically equally susceptible to *P. debaryanum* under the conditions of these experiments. A comparison of results secured with the standard Pythium line confirms this conclusion.

Some of the *Pythium debaryanum* lines were used in miscellaneous small experiments with other coniferous seedlings. This fungus caused 26 out of 30 seedlings of *Abies nobilis* Lind. to damp-off in an experiment in which 1 out of 10 control seedlings damped-off. Because of the difficulty of germinating the Abies seeds it was impossible to conduct other or larger experiments. For the same reason only small experiments were conducted with *Pinus caribaea* Morel. There was no damping-off among the 20 control seedlings, although 18 out of the 30 inoculated ones damped-off. One out of 10 control seedlings of *Pseudotsuga taxifolia* (Lam.) Brit. and 15 out of 20 inoculated ones damped-off. The above tests are purely preliminary but seem to indicate that *Pythium debaryanum* can cause damping-off of *A. nobilis*, *Pinus caribaea*, and *Pseudotsuga taxifolia*. The first two are new hosts for *P. debaryanum*, and heretofore it had not been definitely proven that the last named is a host for it.

Two small test tube experiments and two pot experiments in which seedlings of *Pinus resinosa* were inoculated at the ground level with three of these *Pythium debaryanum* lines confirmed the results secured with the same lines by the platform method.

### Other Phycomycetes

The results secured by inoculating seedlings of *Pinus resinosa* and *P. banksiana* with miscellaneous Phycomycetes by the platform method are also given in Table II.

#### RHEOSPORANGIUM APHANIDERMATUS

The two available lines of *Rheosporangium aphanidermatus* were very parasitic to both species, being approximately as virulent as *Pythium debaryanum* itself, and decidedly more virulent than most of the other fungi. On the whole it was more active when grown on rice mush than when grown on corn meal agar (fig. 2).

#### MUCOR RACEMOSUS

This species was tested on only 20 seedlings in an experiment in which the standard fungi were less virulent than the average, and caused no disease at all. An unknown Phycomycete, which formed only small irregular chlamydospores, caused no disease and the spiny Pythium, which has been referred to the species *artotrogus* caused no appreciable damping-off. In Hartley's (*3*) experiments *P. artotrogus* caused somewhat more damping-off than in the writer's experiments.

#### PYTHIACYSTIS CITROPHTHORA

To avoid the danger of establishing this exotic form in the eastern United States, it was used in a small test-tube

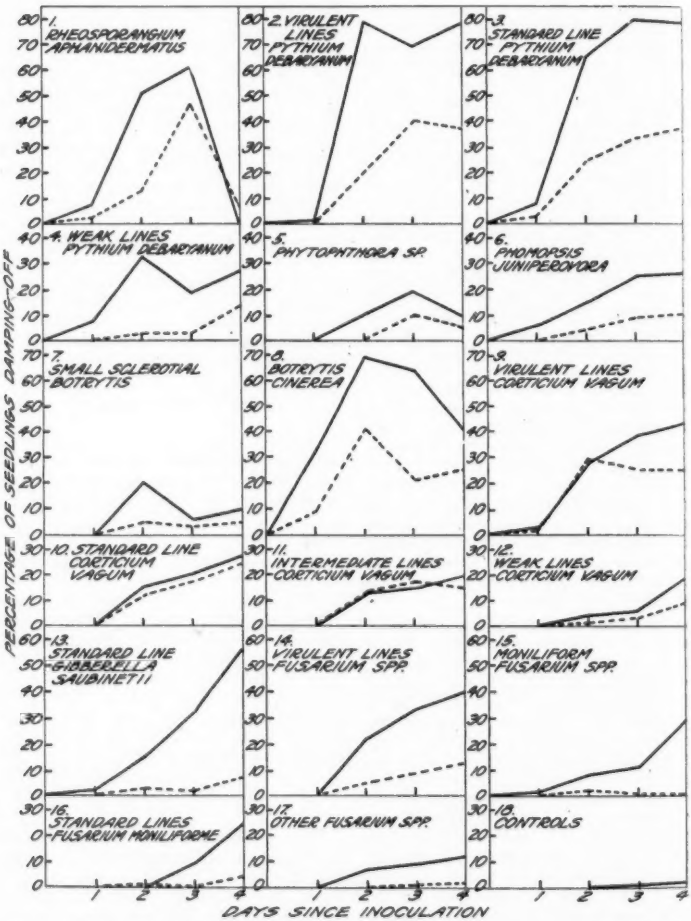

1. RHEOSPORANGIUM APHANIDERMATUS
2. VIRULENT LINES PYTHIUM DEBARYANUM
3. STANDARD LINE PYTHIUM DEBARYANUM
4. WEAK LINES PYTHIUM DEBARYANUM
5. PHYTOPHTHORA SP.
6. PHOMOPSIS JUNIPEROVORA
7. SMALL SCLEROTIAL BOTRYTIS
8. BOTRYTIS CINEREA
9. VIRULENT LINES CORTICIUM VAGUM
10. STANDARD LINE CORTICIUM VAGUM
11. INTERMEDIATE LINES CORTICIUM VAGUM
12. WEAK LINES CORTICIUM VAGUM
13. STANDARD LINE GIBBERELLA SAUBINETII
14. VIRULENT LINES FUSARIUM SPP.
15. MONILIFORM FUSARIUM SPP.
16. STANDARD LINES FUSARIUM MONILIFORME
17. OTHER FUSARIUM SPP.
18. CONTROLS
PERCENTAGE OF SEEDLINGS DAMPING-OFF
DAYS SINCE INOCULATION

FIG. 2.—Diagram showing time elapsing between inoculation and the appearance of damping-off in *Pinus resinosa* seedlings. The percentage of seedlings damped-off each day is based upon the number of seedlings living at the end of the preceding day. The solid line ——— indicates that rice mush was used as the substratum for the inoculum; the broken line - - - - - indicates that corn-meal agar inoculum was used. Only those experiments are reported in which both kinds of inoculum were tested simultaneously. The standard fungi whose effect is shown in graphs 3, 10, 13, and 16 are those lines used in all tests for the sake of comparison; the results with these are not included in the other graphs. The following graphs represent results with more than one line: No. 1, 2 lines; No. 2, 7; No. 4, 3; No. 5, 3; No. 6, 4; No. 7, 2; No. 8, 6; No. 9, 10; No. 11, 8; No. 12, 8; No. 14, 5; No. 15, 11; No. 16, 2; No. 17, 26. Each of the ordinary lines, with some unimportant exceptions, was tested on 20 seedlings with each kind of inoculum and the standard lines (except Gibberella) on 130 or more seedlings on each inoculum. The controls included approximately 1,000 seedlings on each inoculum

experiment with *Pinus resinosa* and caused 100 per cent damping-off compared with 7 per cent in the control plants. This confirms an earlier greenhouse experiment in which inoculations with this fungus at the surface of the soil killed all the seedlings. This is a new host for Pythiacystis.

### PHYTOPHTHORA FAGI

Seedlings of *Pinus resinosa* were inoculated with *Phytophthora fagi* in three small test-tube experiments. The results are given in Table III.

TABLE III.—*Damping-off of seedlings of Pinus resinosa caused by Pythium debaryanum and Phytophthora fagi*

| | First experiment, per cent of damping-off | Second experiment, per cent of damping-off | Third experiment, per cent of damping-off |
|---|---|---|---|
| Controls | 7 | 6 | 6 |
| Pythium debaryanum | 80 | 100 | 100 |
| Phytophthora fagi | 50 | 47 | 67 |

### PHYTOPHTHORA CACTORUM

This species which Rosenbaum (7) combines with *P. fagi* caused practically no damping-off, but was used only in experiments in which, as is shown by results with the standard fungi, conditions were less favorable than usual for damping-off. Like *P. fagi*, it was obviously less virulent than *Pythium debaryanum*. Further comparative results with it are greatly to be desired.

### PHYTOPHTHORA SPECIES

Three lines of a Phytophthora species isolated from pine caused 23 per cent, 43 per cent, and 54 per cent damping-off in the above test-tube experiment in which *P. fagi* caused 67 per cent. In a single experiment one line caused 100 per cent of the seedlings to damp-off. All three lines caused some damping-off of both *Pinus resinosa* and *P. banksiana* with the platform method of inoculation (Table II).

Obviously *Phytophthora cactorum*, *P. fagi* and the pine Phytophthora are less virulent than *Pythium debaryanum*. They are kept separate in this paper because of the need of more extensive taxonomic study and because of the difference in results.

### CORTICIUM VAGUM (RHIZOCTONIA SOLANI KÜHN)

The results of inoculating *Pinus resinosa*, *P. banksiana* and *Picea engelmanni* with *Corticium vagum* lines are given in Table IV. The Corticium lines exhibited considerable variability, and tests of the same lines at different times did not agree very well. One possible explanation of this may be the fact that most of the lines were used in two large experiments, one when it was very hot and the other when it was very cold. This suggests the theory that the virulence of *Corticium vagum* may be closely associated with temperature. Taken as a whole, the *Corticium vagum* lines did not kill half of the inoculated seedlings. However, the most virulent third killed three-quarters of them. In other words, they were more virulent than the standard lines of Corticium or Fusarium, but less virulent than the standard Pythium line. The lines isolated from coniferous hosts were apparently no more virulent from those isolated from other hosts.

The difference between the results secured from the use of corn-meal agar and rice mush as substrata for *Corticium vagum* was comparatively little. In slightly more than half of the units the lines growing on agar caused as much or more damping-off than the same lines growing on rice. Corn-meal agar seemed to be a better substratum for Corticium than for any other fungus tested (fig. 2). A comparison of the lines exclusive of the standard one (No. 147) used on all three hosts gives the following results: For *Pinus resinosa*, 55 per cent damping-off; for *P. banksiana*, 49 per cent, and for *Picea engelmanni*, 24 per cent. A comparison of all the lines used on *P. banksiana* with the same ones on *P. resinosa* gave, respectively, 50 per cent and 49 per cent damping-off. From these results one would assume that the two pine species are of approximately equal susceptibility, while the spruce is less susceptible. However, further experiments are necessary to establish relative susceptibility.

Two preliminary tests in which the seedlings were inoculated with several Corticium lines at the soil surface in general confirmed the results secured by the platform method.

Two lines of *Corticium vagum* killed 7 out of 20 seedlings of *Pinus caribaea* in an experiment in which none of the 20 control seedlings damped-off. This is a new host for Corticium.

TABLE IV.—*Damping-off of coniferous stems caused by Corticium vagum*

| Tested fungi | Number of lines tested | | | Number of seedlings inoculated | | | | Per cent of seedlings killed | | | | | Average of corrected percentages |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pinus resinosa | | | | Pinus resinosa | | | | | |
| | On Pinus resinosa | On Pinus banksiana | On Picea engelmanni | Agar inoculum | Rice inoculum | Pinus banksiana | Picea engelmanni | Agar inoculum | Rice inoculum | Pinus banksiana | Picea engelmanni | Average | |
| Corticium vagum: | | | | | | | | | | | | | |
| All lines | 30 | 23 | 12 | 680 | 730 | 250 | 140 | 36 | 46 | 45 | 36 | 41 | 39 |
| Lines isolated from coniferous hosts | 23 | 17 | 10 | 550 | 580 | 190 | 130 | 37 | 44 | 39 | 36 | 39 | 37 |
| Lines isolated from other hosts | 7 | 6 | 2 | 130 | 150 | 60 | 10 | 30 | 51 | 63 | 0 | 38 | 41 |
| Line No. 147 | 1 | 1 | 1 | 130 | 160 | 30 | 30 | 44 | 58 | 37 | 77 | 54 | 61 |
| Reisolations of line No. 147 | 4 | 3 | 2 | 70 | 70 | 30 | 20 | 71 | 50 | 40 | 5 | 42 | 35 |
| Line No. 213 | 1 | 1 | ---- | 20 | 20 | 10 | ---- | 70 | 75 | 100 | ---- | 82 | 85 |
| Reisolations of line No. 213 | 2 | 2 | 1 | 40 | 40 | 20 | 10 | 55 | 68 | 55 | 60 | 60 | 62 |
| Line No. 230 | 1 | 1 | ---- | 20 | 20 | 10 | ---- | 50 | 100 | 100 | ---- | 83 | 81 |
| Reisolations of line No. 230 | 3 | 3 | 1 | 60 | 60 | 30 | 10 | 40 | 50 | 33 | 30 | 39 | 43 |
| Line No. 240 | 1 | 1 | 1 | 20 | 20 | 10 | 10 | 75 | 100 | 100 | 90 | 91 | 93 |
| Rhizoctonia potomacensis [a] | 1 | 1 | 1 | 20 | 20 | 10 | 10 | 5 | 35 | 30 | 0 | 18 | 18 |
| Controls | ---- | ---- | ---- | 975 | 1, 225 | 450 | 300 | 0. 1 | 2. 5 | 0. 7 | 0. 3 | 1— | ---- |

[a] Only a line of *Corticium vagum*, accordi ng to H. A. Edson, who furnished it.

TABLE V.—*Damping-off of coniferous stems caused by Fusarium spp.*

| Tested fungi | Number of lines tested | | | Number of seedlings inoculated | | | | Per cent of seedlings killed | | | | | Average of corrected percentages |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Pinus resinosa | | | | Pinus resinosa | | | | | |
| | On Pinus resinosa | On Pinus banksiana | On Picea engelmanni | Agar inoculum | Rice inoculum | Pinus banksiana | Picea engelmanni | Agar inoculum | Rice inoculum | Pinus banksiana | Picea engelmanni | Average | |
| Fusarium sporotrichioides | 1 | 1 | 1 | 20 | 20 | 10 | 10 | 25 | 100 | 90 | 80 | 74 | 83 |
| Fusarium discolor sulphureum | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 50 | 90 | ---- | ---- | 70 | 54 |
| Fusarium arthrosporioides | 1 | 1 | ---- | 20 | 20 | 10 | ---- | 15 | 65 | 40 | ---- | 40 | 52 |
| Fusarium trichothecioides | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 40 | 35 | ---- | ---- | 38 | 20 |
| Gibberella saubinetii | 2 | 1 | 1 | 80 | 90 | 30 | 30 | 8 | 56 | 50 | 27 | 35 | 27 |
| Fusarium hyperoxysporum | 1 | 1 | ---- | 20 | 20 | 10 | ---- | 0 | 55 | 50 | ---- | 35 | 32 |
| Fusarium sp. between roseum and discolor groups | 1 | 1 | 1 | 20 | 20 | 10 | 10 | 0 | 55 | 20 | 40 | 29 | 28 |
| Fusarium moniliforme species and section: | | | | | | | | | | | | | |
| All lines | 13 | 11 | 6 | 380 | 410 | 130 | 80 | 5 | 41 | 28 | 26 | 25 | 19 |
| Lines isolated from coniferous hosts | 8 | 7 | 3 | 280 | 310 | 90 | 50 | 7 | 41 | 34 | 26 | 27 | 20 |
| Lines isolated from other hosts | 5 | 4 | 3 | 100 | 100 | 40 | 30 | 0 | 40 | 18 | 7 | 16 | 15 |
| Nectria ipomoeae | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 10 | 40 | ---- | ---- | 25 | 18 |
| Fusarium martiella section | 1 | 1 | ---- | 20 | 20 | 10 | ---- | 0 | 5 | 70 | ---- | 25 | 24 |
| Unidentified Fusarium spp | 2 | ---- | ---- | 40 | 40 | ---- | ---- | 3 | 40 | ---- | ---- | 22 | 18 |
| Fusarium solani group | 3 | 1 | ---- | 60 | 60 | 10 | ---- | 0 | 17 | 50 | ---- | 22 | 19 |
| Fusarium orthoceras? | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 10 | 30 | ---- | ---- | 20 | 40 |
| Fusarium martii | 1 | 1 | ---- | 20 | 20 | 10 | ---- | 5 | 15 | 20 | ---- | 13 | 18 |
| Fusarium culmorum group | 3 | 3 | 1 | 60 | 60 | 30 | 10 | 3 | 45 | 0 | 0 | 12 | 12 |
| Fusarium radicicola | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 10 | 10 | ---- | ---- | 10 | 4 |
| Fusarium elegans group | 8 | 2 | 1 | 150 | 170 | 20 | 10 | 1 | 19 | 15 | 0 | 9 | 10 |
| Fusarium avenaceum | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 0 | 15 | ---- | ---- | 8 | 12 |
| Fusarium coeruleum | 1 | ---- | ---- | 20 | 30 | ---- | ---- | 0 | 13 | ---- | ---- | 7 | 2 |
| Fusarium acuminatum | 3 | 1 | ---- | 60 | 60 | 10 | ---- | 2 | 7 | 10 | ---- | 6 | 4 |
| Fusarium vasinfectum | 2 | 1 | ---- | 40 | 40 | 10 | ---- | 3 | 13 | 0 | ---- | 5 | 7 |
| Fusarium discolor section | 3 | 1 | ---- | 40 | 60 | 10 | ---- | 0 | 5 | 10 | ---- | 5 | 5 |
| Fusarium ventricosum | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 0 | 0 | ---- | ---- | 0 | 0 |
| Fusarium oxysporum | 1 | 1 | ---- | 20 | 20 | 10 | ---- | 0 | 0 | 0 | ---- | 0 | 0 |
| Fusarium eumartii | 1 | ---- | ---- | 20 | 20 | ---- | ---- | 0 | 0 | ---- | ---- | 0 | 0 |
| Controls | ---- | ---- | ---- | 975 | 1, 225 | 450 | 300 | 0. 1 | 2. 5 | 0. 7 | 0. 3 | 1 | ---- |

### Fusarium spp.

The results of inoculation with various species of Fusarium are given in Table V. *F. sporotrichioides* and *F. discolor sulphureum* were very parasitic, comparing favorably in virulence with the more parasitic Pythium and Corticium lines. The species of the moniliform section of Fusarium ranged in virulence from the very parasitic to the almost nonparasitic forms. *F. arthrosporioides, F. hyperoxysporum, F. trichothecioides,* and *Gibberella saubinetii* were all moderately parasitic. *F. eumartii, F. oxysporum,* and *F. ventricosum* were definitely nonparasitic under the conditions of these experiments. *F. acuminatum, F. avenaceum. F. coeruleum,* certain lines of the *F. discolor* groups, *F. martii, F. radicicola* and *F. vasinfectum* were either nonparasitic or at times very doubtfully parasitic. It is not probable that any of these would cause much trouble under nursery conditions. Certain lines of the *F. culmorum* group, *F. martiella, Nectria ipomoeae, F. orthoceras, F. solani* and some unidentified *Fusarium* spp. showed evidence at times of being intermediate between the very parasitic and the nonparasitic groups. Further experiments are absolutely necessary before the ability of either of these last two classes to attack conifers is established.

In no case where there was a definite measurable degree of parasitism was Fusarium so active on corn-meal agar inoculum as on rice mush. (Table V and fig. 2.) In one small experiment with *Pinus caribaea* in which none of the 10 control seedlings died and in which 4 of the 10 seedlings inoculated with *Pythium debaryanum* damped-off, *Fusarium moniliforme* caused 3 out of 10 seedlings to damp-off. This purely preliminary experiment indicates *P. caribaea* as a new host for *F. moniliforme.*

### Botrytis spp.

Table VI gives the results of inoculating *Pinus resinosa* and *P. banksiana* with *Botrytis* spp. The two available small sclerotial forms of Botrytis were either very slightly parasitic or entirely nonparasitic on both pine hosts. When grown on rice mush one of them seemed fairly parasitic to *P. resinosa.* It is probable, however, that this specialized parasite under ordinary conditions would be of no importance on coniferous seedlings.

The large sclerotial forms of Botrytis were all much alike and were all of the type which has previously gone under the name of *Botrytis cinerea.* It is considered best to continue the use of that generally known name until there is published warrant for change or segregation. These large sclerotial forms were more parasitic than the standard Fusarium line. They were always as destructive, and in most cases more so, than the standard Corticium line, and in all but one experiment, fully equaled the standard *Pythium debaryanum.* It is a less important parasite for coniferous seedlings than the latter, because in only a few cases has it been isolated from damped-off coniferous seedlings.

A comparison of the damping-off caused by the same lines of *Botrytis cinerea* on *Pinus resinosa* and *P. banksiana,* gave in both cases a result of 94 per cent, both species appearing highly and equally susceptible to them. There is a slight difference between

Table VI.—*Damping-off of coniferous stems caused by miscellaneous fungi*

| Tested fungi | Number of lines tested | | Number of seedlings inoculated | | | Per cent of seedlings killed | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | On Pinus resinosa | On Pinus banksiana | Pinus resinosa | | Pinus banksiana | Pinus resinosa | | Pinus banksiana | Average | Average of corrected percentages. |
| | | | Agar inoculum | Rice inoculum | | Agar inoculum | Rice inoculum | | | |
| Botrytis cinerea: | | | | | | | | | | |
| All lines | 6 | 4 | 130 | 130 | 70 | 68 | 95 | 94 | 86 | 86 |
| Lines isolated from coniferous hosts | 1 | 1 | 20 | 20 | 20 | 50 | 90 | 80 | 73 | 68 |
| Lines isolated from other hosts | 5 | 3 | 110 | 110 | 50 | 72 | 96 | 100 | 89 | 92 |
| Phomopsis juniperovora | 4 | 4 | 70 | 90 | 80 | 21 | 53 | 66 | 47 | 35 |
| Cephalothecium roseum | 1 | 1 | 20 | 20 | 20 | 30 | 45 | 35 | 37 | 14 |
| Small sclerotial Botrytis | 2 | 2 | 40 | 40 | 40 | 13 | 32 | 5 | 17 | 12 |
| Pestalozzia funerea? | 1 | 1 | 20 | 20 | 20 | 0 | 20 | 0 | 7 | 7 |
| Verticillium sp | 1 | 1 | 20 | 20 | 20 | 0 | 0 | 0 | 0 | 0 |
| Thielavia basicola | 1 | 1 | 10 | 10 | 20 | 0 | 0 | 0 | 0 | 0 |
| Controls | | | 975 | 1,225 | 450 | 0.1 | 2.5 | 0.7 | 1 | |

the above figure and the ones given in Table VI because more lines were used on *P. resinosa* than on *P. banksiana*. Isolation from a coniferous host did not increase virulence of *Botrytis cinerea*.

In only 6 per cent of the tested cases was Botrytis more virulent when grown on corn-meal agar than when grown on rice mush. The virulence was, however, equal in 41 per cent of the cases. The different lines exhibited more variability when grown on agar than when grown on mush (fig. 2). Four lines showed the same virulence when the inoculum was placed at the ground level as when it was placed on platforms.

### Miscellaneous Fungi

Table VI gives the results of inoculating *Pinus resinosa* and *P. banksiana* with miscellaneous fungi by the platform method.

All lines of *Phomopsis juniperovora* showed some evidence of parasitic ability. There is some slight evidence that *Pinus banksiana* is slightly more susceptible than *P. resinosa* to this species. Hartley (*2*) and his associates previously found the species unable to cause damping-off in soil inoculations. There is a possibility that the reason for this is the inability of the fungus to maintain itself in the soil saprophytically until the seed has sprouted.

*Cephalothecium roseum* showed some slight evidence, which needs confirmation, of ability to cause damping-off of both species. *Thielavia basicola*, *Pestalozzia funerea*? and an unidentified *Verticillium* sp. caused no damping-off.

## TIME ELAPSING BETWEEN INOCULATION AND APPEARANCE OF DAMPING-OFF

As stated above, all experiments were continued for four days. Figure 2 gives a set of graphs comparing the daily damping-off of *Pinus resinosa* seedlings when inoculated with various groups of fungi grown on rice mush and corn-meal agar. The percentages for each day are based upon the number of seedlings living at the end of the preceding day.

*Pythium debaryanum*, *Rheosporangium aphanidermatus*, *Phomopsis juniperovora* and the more virulent *Corticium vagum* lines caused the collapse of some seedlings within 24 hours from the placing of the inoculum, while *Botrytis cinerea* caused almost one-third of the seedlings to damp-off during the same period. With the exception of Phomopsis, these same fungi caused about the same percentage of *P. banksiana* seedlings to damp-off by the end of the first day. There was a slight amount of damping-off caused by *Fusarium* spp. during the same period. By the end of the first day *P. debaryanum* caused a somewhat larger percentage of the *Picea engelmanni* seedlings to damp-off.

At the end of the fourth day for most of the Fusarium and Corticium groups the percentage of damping-off was increasing (fig. 2). This probably means that the experiments were closed before some of these lines had showed their maximum parasitic ability. This fact may explain in part why in these experiments Corticium seemed less virulent than formerly (*3*). Most of the other fungi seemed to have reached their maximum parasitic ability by the end of three days.

*Botrytis cinerea*, *Rheosporangium aphanidermatus*, and *Pythium debaryanum* all grow very rapidly on artificial media. This may explain in part why damping-off symptoms appeared so soon on seedlings inoculated with them. However, Phomopsis grows slowly. The first three genera are also all very virulent parasites. It is rather surprising in view of their reputation for toxicity that the more parasitic of the *Fusarium* spp. were so much slower than Pythium in producing a visible effect on the seedlings.

Ten Fusarium lines and a few Corticium lines which always gave negative results were omitted from the graphs. No damping-off was noticeable in the controls until the end of three days.

## DISCUSSION OF RESULTS

Lines of *Pythium debaryanum*, *Rheosporangium aphanidermatus*, *Corticium vagum*, *Botrytis cinerea*, and a few of the numerous species of Fusarium were all able to cause considerable damping-off. *Pythium debaryanum*, the very similar Rheosporangium, and *Botrytis cinerea* seem able to damp-off approximately the same percentage of the inoculated seedlings. However, in view of the fact that Hartley and his assistants (*2*) found Botrytis causing damping-off only in the laboratory and never in the nursery bed, and have never found Rheosporangium occurring naturally in coniferous seedlings, less importance is attached to inoculation results with them. They are very active parasites and are potentially capable of causing great damage, if present under nursery conditions.

Under the conditions of the writer's experiments, the *Pythium debaryanum* lines showed a smaller virulence range than did the same ones when used in soil inoculation experiments by Hartley (*2*). There are two possible explanations for this: (1) Some lines may have formerly been considered nonparasitic because of their inability to live saprophytically in the soil until the seeds had germinated, and (2) lines really weak may have seemed strongly parasitic in the writer's tests because of the heavy inoculum. The same may be true also of other genera.

For unexplained reasons, *Corticium vagum* exhibited far more variability than it did in Hartley's (*2*, *3*) experiments.

Few of the *Fusarium* species exhibited decidedly high or constant parasitic ability, but as a whole the genus appears able to cause considerable damage.

The other tested fungi besides *Pythium debaryanum*, *Corticium vagum*, *Botrytis cinerea* and *Fusarium* spp. are probably unimportant in the field because of the infrequency of their occurrence in coniferous nurseries even though some lines tested in these experiments were rather virulent.

The fact that the virulence and variability of the parasites depend to a certain extent on the medium used as inoculum, suggests that the virulence of the parasites in the soil, or at least their ability to maintain themselves saprophytically there until seeds had germinated, is largely dependent upon the amount and quality of the available food supply.

In order definitely to establish the relative susceptibility of the various hosts, tests should be conducted in which all the hosts are inoculated in the same experiment. However, it is to be expected that the hosts would be of approximately equal virulence in these experiments because Hartley (*3*) reported *Pinus resinosa*, *P. banksiana*, and *Picea engelmanni* as highly susceptible to damping-off under field conditions. It is probable that the fungi which proved themselves only moderately parasitic under the extremely artificial conditions of these experiments would do little or no damage under nursery conditions.

## SUMMARY

(1) Direct inoculations of stems without bringing the inoculum in contact with the soil were conducted in the greenhouse of the United States Department of Agriculture at Washington, D. C. with seedlings of *Pinus resinosa*, *P. banksiana*, and *Picea engelmanni*. Over one hundred different fungous lines, most of which were *Pythium debaryanum*, *Corticium vagum*, and *Fusarium* spp., were tested.

(2) The virulence and variability of the different fungi depend to some extent on the substratum on which they are grown.

(3) The most virulent parasites in these experiments were *Pythium debaryanum*. *Botrytis cinerea*, *Rheosporangium aphanidermatus* and *Fusarium sporotrichioides*.

(4) The time elapsing between inoculation and the appearance of damping-off was especially short for the virulent swift-growing fungi.

## LITERATURE CITED

(1) DAVENPORT, C. B.
1904. STATISTICAL METHODS; WITH SPECIAL REFERENCE TO BIOLOGICAL VARIATION. Ed. 2, 223 p. New York.
(2) HARTLEY, C., MERRILL, T. C., and RHOADES, A. S.
1918. SEEDLING DISEASES OF CONIFERS. Jour. Agr. Research 15: 521–558, illus.
(3) ———.
1921. DAMPING-OFF IN FOREST NURSERIES. U. S. Dept. Agr. Bul. 934, 99 p., illus.
(4) RATHBUN, A. E.
1921. METHODS OF DIRECT INOCULATION WITH DAMPING-OFF FUNGI. Phytopathology 11: 80–84, illus.
(5) ———.
1922. ROOT ROT OF PINE SEEDLINGS. Phytopathology 12: 213–220, illus.
(6) ———.
1923. DAMPING-OFF OF TAPROOTS OF CONIFERS. Phytopathology 13: 385–391.
(7) ROSENBAUM, J.
1917. STUDIES OF THE GENUS PHYTOPHTHORA. Jour. Agr. Research 8: 233–276, illus.
(8) SPAULDING, P.
1914. THE DAMPING-OFF OF CONIFEROUS SEEDLINGS. Phytopathology 4: 73–88, illus.

# ROOT ROT OF THE GRAPEVINE IN MISSOURI CAUSED BY CLITOCYBE TABESCENS (SCOP.) BRES.[1, 2]

By ARTHUR S. RHOADS

*Assistant Plant Pathologist, Agricultural Experiment Station of the University of Florida; formerly Pathologist, Missouri State Fruit Experiment Station*

## INTRODUCTION

Shortly after the writer had assumed his duties at the Missouri State Fruit Experiment Station in 1922 his attention was directed to reports received from several localities of the destruction of grapevines caused by root rot. The first opportunity to investigate this disease was afforded by a trip to Knobview, Mo., on May 30, 1922, where, in the vineyard from which the trouble was reported, a number of vines that had recently died were carefully dug up. All exhibited the same symptoms, namely, an abundant growth of characteristic whitish to isabelline mycelial sheets or mats occurring between the layers of the innner bark and between the bark and the wood of the roots and root crown, occasionally accompanied by black rhizomorphic strands. These symptoms indicated that the disease was caused by a mushroom root rot, although no fruiting bodies could be found to verify the supposition. Cultures and further field studies were made which afforded a basis for the conclusions herein reported.[3]

## HISTORICAL REVIEW

Although grapevines are known to be attacked by several different root rotting fungi, especially when the root systems have become weakened or injured, comparatively few investigations have been made in this country of the resulting diseases.

Root rot of the grapevine has long been known in Europe, where it has been investigated extensively. In France it is called "blanc des racines" or, more commonly, "pourridié;" in Germany "Weinstock-fäule;" and in Australia and some other countries "white-rot." According to Verge (*56*)[4] the cause of pourridié in Europe is attributed to several of the higher fungi, among them being *Armillaria mellea* (Fries) Quél., *Dematophora necatrix* Hartig, *Vibrissea* (*Roesleria*) *hypogaea* Ch. Richon and Le Monnier, and sometimes *Psathyrella ampelina* Foex and Viala. In the United States the disease has sometimes been attributed to these fungi and also to *Phymatotrichum* (*Ozonium*) *omnivorum* (Shear) Duggar. Most American writers, however, have generally believed that the disease in this country is caused by *Armillaria mellea* and *Dematophora necatrix* (*51, p. 172–174*), although as a rule on assumption rather than from definite evidence.

Root rot of the grapevine was first reported in this country in Missouri, Scribner (*46, p. 137*) stating that it was discovered in a vineyard at Bushberg, about 25 miles south of St. Louis, by the eminent French viticulturist Viala, in 1887, while the two were making a tour of the principal grape-growing regions of the United States. The same author also states (*47, p. 12; 48, p. 64*) that they found the disease later in northeastern Texas and again in Napa Valley, Calif. He records the fact that he has seen a number of vines nearly or quite dead from the same disease in the vicinity of Knoxville, Tenn.

In 1892 Pierce (*38 p., 153–161*), discussing the relation of root-attacking fungi to the California vine disease, states that Dematophora, although found in several places in the United States, had not been seen in California; that *Armillaria mellea* has been reported in northern California; and that *Vibrissea hypogaea* was found in

[1] Received for publication May 19, 1924; issued April, 1925.

[2] Commonly known in this country under the names *Clitocybe monadelpha* (Morg.) Sacc., *Armillaria mellea exannulata* Peck, *Clitocybe parasitica* Wilcox, and *Monadelphus caespitosus* (Berk.) Murrill.

[3] The writer gratefully acknowledges helpful advice and suggestions concerning the taxonomic treatment of the fungus under discussion from Dr. E. A. Burt of the Missouri Botanical Garden, Dr. L. O. Overholts of the Pennsylvania State College, and C. G. Lloyd of the Lloyd Library and Museum at Cincinnati. Grateful acknowledgment is made of the invaluable services rendered by the libraries of the Missouri Botanical Garden and the U. S. Department of Agriculture.

[4] Reference is made by number (italic) to "Literature cited," p. 364.

the Santa Ana Valley on the roots of vines which had been brought from Missouri. He also reports the occurrence of a species of Graphium infesting diseased vine roots in the Santa Ana Valley but thinks that it bore no relation to the cause of the disease in question.

Between this date and 1900 a few additional reports were made of grapevine root rot. McCarthy (*25, p. 122*), discussing the disease in North Carolina, and Starnes (*50, p. 282*), in Georgia, both arbitrarily attribute it to *Dematophora necatrix* and *Armillaria* (*Agaricus*) *mellea*, merely because the same disease in Europe was commonly attributed to attacks by these fungi. Underwood and Earle (*54, p. 272*), discussing root rot of the grapevine in Alabama, state that it seems to be identical with the disease known as "pourridié" in France. They believe that it is quite prevalent and often does serious damage, as evidence of which they mention the fact that out of the 584 vines in the station vineyard all but 83 showed evident signs of the disease, and that many died during the late summer and fall. They note that the character of the soil apparently has much to do with the prevalence of the disease, that vines may live many years while more or less affected by it, and that some varieties are evidently much more resistant than others. Earle and Austin (*14*), writing four years later, give a much more detailed account of grapevine root rot in Alabama and question the earlier statement of Underwood and Earle (*54, p. 272*) that this disease is the same as the "pourridié" of the French, which they say is caused by *Dematophora necatrix*. Repeated attempts to isolate and culture the organism causing the whitish mycelial growth under the bark of diseased roots were unsuccessful and further observations led Earle and Austin to conclude that the disease works much more slowly than the European root rot. These authors state that the mycelial whitening can often be found on old Scuppernong vines and on wild grapevines in the woods, although these are seldom if ever killed by it, and that its presence on the roots of cultivated vines is by no means a sure sign of immediate death. From an examination of the statistics on grape planting at the Alabama station these authors conclude that the disease is a very serious one. They state that of the 651 vines alive or planted in 1894, a loss of 483, or 75 per cent, was sustained in six years and, although there was no proof that all of these were affected by root rot, they believe that the greater part of them died from this cause. These authors conclude that the Herbemont and Rulander varieties are immune and that the Delaware is very resistant to mortality from root rot.

In 1901 Wilcox (*58*) described a rhizomorphic root rot of fruit trees which was causing widespread destruction of orchards in Oklahoma and adjacent States. He demonstrates that this disease is caused by a species of Clitocybe, which he describes as a new species, *Clitocybe parasitica*. In addition to its occurrence on fruit trees, he finds the fungus to be a common parasitic and saprophytic form on four species of oaks in Oklahoma. A rhizomorphic root rot, which Wilcox assumes was caused by this species of Clitocybe, is reported as occurring also in Texas, Missouri, southern Illinois, Indiana, and to some extent in Ohio, Georgia, California, and Oregon. Except for a few references in the bibliography appended, Wilcox makes no mention whatever of this disease on the grapevine, which, in some cases, undoubtedly is caused by the same fungus as that with which he was working.

Walker (*57, p. 30*) and Hewitt and Hayhurst (*21, p. 424*) reported that root rot of fruit trees is widespread in Arkansas and causes serious damage in some localities. The first author says that, "besides the apple it affects probably all of the commonly cultivated fruit trees, the grape as well as a number of forest trees," and states that the disease is caused by toadstool fungi, "two of the forms concerned being *Clitocybe parasitica* and *Armillaria mellea*."

Duggar (*13, p. 471*) mentioned the abundant occurrence of *Clitocybe parasitica* at Columbia, Mo., during favorable seasons on roots of hickory and other deciduous trees, but failed to observe its occurrence in orchards, despite special effort to find it. The suggestion by some observers that *Armillaria mellea* is responsible for the root rot of fruit trees attributed to *Clitocybe parasitica* is refuted by Duggar, who states that he has never detected this fungus associated with the typical disease in Missouri. Although *Armillaria mellea* may occur in Missouri, and in this case be responsible for some of the root rot reported, the writer has never seen it in the State even in forests where in favorable seasons *Clitocybe parasitica* often abounds.

Butler (*8, p. 24–29*), in his account of root rot of grapevines in California, describes a slow and a rapid form of

the disease, but contributes nothing to the identity of the causal organisms involved. The vineyard in which an exceptionally severe form of root rot was observed was planted on a slope shortly after the land had been cleared of its oak timber, and the lower part was poorly drained. From his experience with California conditions, the writer would attribute the form of root rot prevailing there to *Armillaria mellea*.

## SYMPTOMS OF THE DISEASE

Often, without any cause apparent to the vineyardist, the vines will exhibit a sickly appearance which becomes quite evident in midsummer, when the demand for water conduction is greatest. At this time the margins of the leaves on diseased vines suddenly turn brown, usually shortly before the ripening of the fruit. Later the leaves may dry up entirely and the vine suddenly die, leaving the fruit to shrivel in the sun (pl. 1), or the crop may mature and the vine linger until fall, being entirely dead at the winter pruning. In other cases only a part of the vine will die, some branches putting forth a feeble growth for two or three seasons longer.

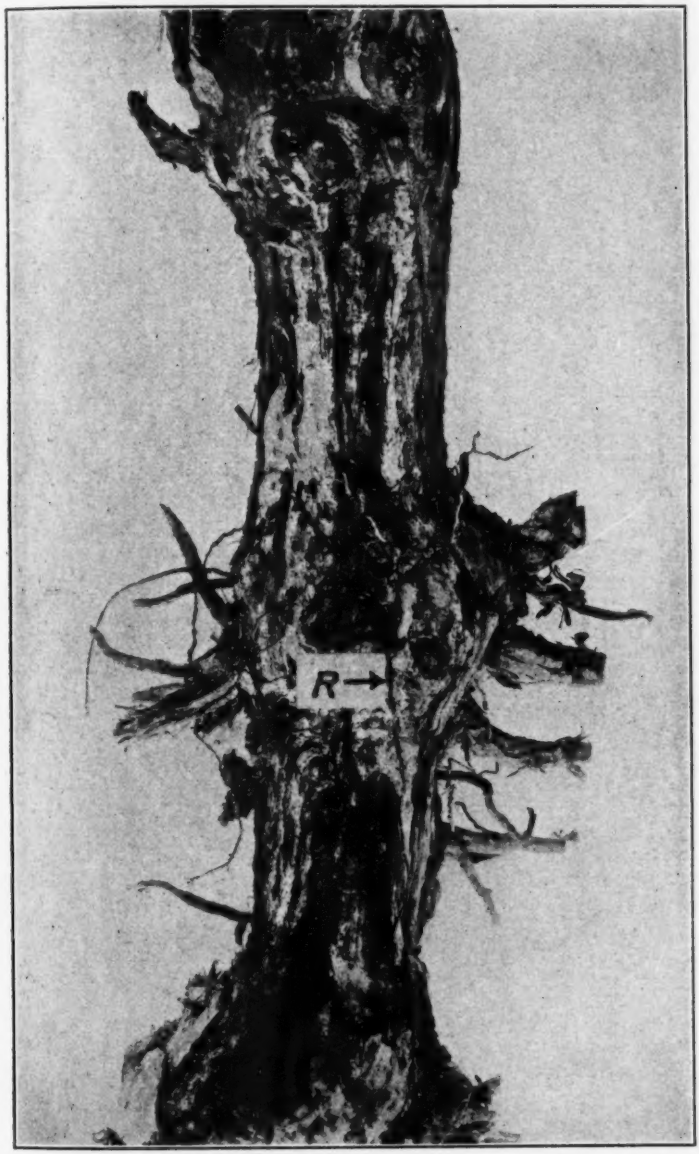
The root crowns and larger roots of diseased vines, when dug up and the outer fibrous bark peeled off, invariably show a whitish to creamy white or isabelline mycelial coating or sheet, the marginal portions of which spread out in a fan-shaped manner, the older parts often forming a feltlike layer of fungous tissue between the outer bark and the wood (pl. 2). Often there is present also a number of more or less flattened, black rhizomorphs. The inner living bark has been killed by the mycelium, which usually can be traced from 1 to 3 inches above the ground line and down to all the larger roots. In well-advanced cases the smaller fibrous roots may be rotted away, although in less advanced cases only the crown and the larger roots may be affected, the smaller ones remaining for the most part healthy. There is comparatively little decay of the wood of the root crown or larger roots until after the death of the vine, in which case transverse sections through the root crown or larger roots exhibit whitish radial streaks of more or less delignified wood.

The course and nature of the decay are essentially the same as those described by Wilcox (*58*) for fruit trees. Within the wood the mycelium attains its greatest development in the medullary rays, probably by reason of the fact that these are centers for the storage of reserve food materials. This great growth of mycelium within the larger medullary rays soon leads to the formation of radial cracks in the wood, which become stuffed with light tan-colored sheets or feltlike mats of mycelium often extending into the pith. This tendency of the mycelium to develop most abundantly within the larger medullary rays explains why the decay first appears as whitish more or less delignified radial streaks. Within the centers of decay the cell walls fail to respond to microchemical tests for lignin and various stages of dissolution are to be seen there. Within the individual cell the dissolution proceeds outward from the laminae bordering on the lumen, the middle lamella or primary layer being the last part to disappear. Contrary to the statement by Wilcox (*58, p. 15*) that the hyphae find their way into the cells only through the pits, the writer finds that they may penetrate the walls regardless of the presence of pits, as is the case in virtually all fungi causing an enzymatic digestion of the wood elements and the consequent decay of the wood.

The mycelial growth through the inner bark is by no means confined to vines in which the foliage has shown signs of disease, but frequently may be found on those that are still making a fairly strong growth and on which the foliage is perfectly healthy. This point can be determined readily in an infected vineyard by making a slicing cut down through the outer bark at the ground line. The presence of the mycelium in the inner bark of the roots is by no means an indication of speedy death, however, for the disease works comparatively slowly and vines may be affected for a number of years before they succumb. Although the writer has no definite figures on this point, it is believed that at least from two to four years are required for this root rot fungus to kill a well-established vine. When the mycelium has progressed sufficiently through the wood and the inner living bark to cut off, either wholly or in part, the water supply, the vine dies more or less suddenly.

During the late summer and autumn, in favorable years, one often finds in vineyards and orchards suffering from root rot clusters of the mushrooms fruiting from the root crowns of vines and fruit trees. These may be found even more abundantly in forests, especially in oak stands (pl. 3).

Concord grapevine killed by Clitocybe root rot. All the leaves and fruit dried up shortly before the harvest. The living leaves showing in the background are on the vine in the next row




Rootstock of Concord vine recently killed by Clitocybe root rot. The outer fibrous bark has been peeled away to show the whitish to buff colored mycelial mat and the branching blackish rhizomorph at the point marked R. The level at the top of the picture was but a short distance below the ground line. Three-fourths natural size

Clusters of sporophores of *Clitocybe tabescens* in woods consisting largely of oak. Hundreds of these clusters of mushrooms occurred in one section of woods, most of them growing from the bases and roots of dead trees and stumps, or from buried wood, while a few others grew from the bases of living trees. The cluster at the left was placed in the field of view to show the character of the under side

## CULTURAL STUDIES

Cultures made from the mycelial sheets on the roots of grapevines affected by root rot invariably yielded a very characteristic, slow-growing organism. Very scant growth of the fungus or none was obtained on cornmeal agar, and cultures on plain agar resulted in but weak growth. A much better growth was secured on prune agar and a very satisfactory growth on both maltose agar (30 gm. maltose per liter) and raisin agar (50 gm. seeded raisins per liter). The growth of the fungus on these different media indicates that it does best on media rich in sugar.

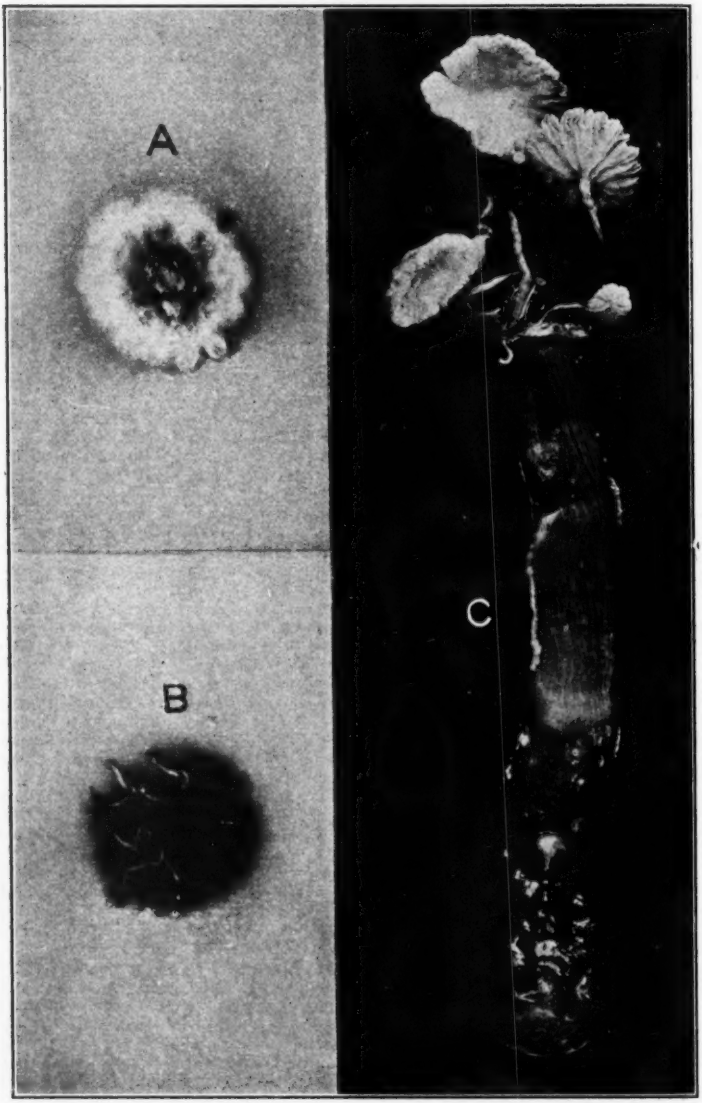
Since this organism develops rhizomorphs that grow down into the agar, it was found highly desirable early in the work to have the culture media as clear as possible in order to best observe the development and morphological characters of these structures. In order to clarify the agar, the whites of two eggs were added to each liter and the whole boiled prior to tubing and sterilization.

The initial mycelial growth was whitish, the marginal growth in most cases being rather sparse and downy-appressed, although in a few instances a rather dense white marginal growth occurred (pl. 4, A). In all cases the mycelial growth eventually became thickened at the center, so that it presented a convex outline, the older portion near the center soon becoming a dirty white and later changing to isabelline or light buff. Often the immediate central part would appear as an irregular, light tan-colored lump. In the case of cultures made on raisin agar the agar invariably became discolored, showing dark brown immediately beneath the mycelial mat and for some distance in advance of it. No such discoloration was noted, however, in any of the cultures on maltose agar. After the lapse of from two to three weeks, rarely longer, there would grow down into the agar, from the central part of the culture, peculiar, tortuous whitish strands, which constitute the beginning of rhizomorph formation (pl. 4, B). The plate cultures invariably dried up before the fungus attained any great development, so that their use was abandoned early in the work. Even tube cultures made in the size of tubes commonly used dried up as a rule before fruiting bodies developed. The best results with this slow-growing organism were obtained by making the cultures in large tubes and flasks. In some of the small-tube cultures a group of little hornlike processes—the primordia of clusters of mushrooms—often developed from the central part of the culture, but never developed further on account of the drying up of the cultures.

On July 19, 1922, the fungus was inoculated on 4-inch lengths of roots of wild grape (*Vitis cordifolia* Michx.), autoclaved in large test tubes containing a small quantity of water. From the mycelial transfer, which was placed on the top of the root length, the mycelium quickly grew down through the inner bark, even to the bases standing in water. At a few points it pierced the bark and developed superficial masses of mycelium, a few light tan-colored nodules forming on the tops.

Just a few days before the fruiting of one of these sets of cultures, the writer, while making a study of the grape diseases at Neosho, Mo., during the picking season, found several clusters of a caespitose species of mushroom developing from the bases of Moore's Early and Concord vines which had been killed by root rot in a badly infected vineyard. Large numbers of clusters of the same fungus were observed also in one section of an oak forest (pl. 2) several miles distant, growing from the bases of dead oak trees and from roots beneath the surface of the ground. One large cluster was also observed growing from the superficial roots of an old silver maple shade tree in Neosho. The morphological characters and spore measurements of these specimens agreed perfectly with those of *Clitocybe tabescens* (Scop.) Bres., of which Morgan's *Agaricus monadelphus*, Wilcox's *Clitocybe parasitica*, and Murrill's *Monadelphus caespitosus* represent the American forms. A representative specimen from both the vineyard and the oak forest was sent to Murrill, who identified both as *Monadelphus caespitosus*.

Upon the writer's return from Neosho he was agreeably surprised to find one of the series of cultures on the lengths of grape roots beginning to develop fruiting bodies. On August 10, a group of hornlike processes appeared on the top of the root length in one of the tubes. By August 17 this had developed into a miniature cluster of mushrooms that was reasonably typical of those collected in the field a few days before. With the lapse of four more days the mushrooms of this cluster had attained their full development (pl. 4, C) and had cast on the side of the tube a print of white




A.—One-month-old Petri dish culture of Clitocybe root rot, isolated from a recently killed vine, on raisin agar. The marginal mycelium is whiter and more superficial than in the case of cultures on other agars used. About 1¼ natural size

B.—Under side of same culture showing the dark-brown discoloration of the agar and the characteristic whitish rhizomorphs beginning to develop beneath the surface of the agar. About 1¼ natural size

C.—Cluster of sporophores of *Clitocybe tabescens* developed in artificial culture made by inoculating a sterilized length of root of *Vitis cordifolia* in a large test tube. This cluster of sporophores developed to maturity and cast a print of white spores on the side of the tube within one month and three days from the time the length of root was inoculated. About natural size

spores typical of *Clitocybe tabescens*. Thus mature fruiting bodies shedding spores were developed within approximately one month from a mycelial transfer.[5]

None of the other cultures of the series on lengths of wild grape roots fruited, some becoming contaminated with molds and others drying up. No rhizomorph formation was noted on any of the lengths of roots, but, when the bark was peeled off, all exhibited the whitish fan-shaped mycelial sheets characteristic of the root rot of grapevines found in the field. The source of the inoculum for this set of cultures was the rotted vine roots collected at Knobview in the early part of the summer, at which time no fruiting bodies were in evidence.

The type of the root rot, including the fan-shaped mycelial sheets and the rhizomorphs, of the vines at Neosho was identical with that of the Knobview vines first studied, and cultures secured from the rotted vine roots bearing sporophores at Neosho agreed in every way with those secured from the Knobview material. Although the writer has had no opportunity to attempt the reproduction of this root-rot disease of grapevines by inoculations with pure cultures of the fungus isolated from infected vines, he is thoroughly convinced that it is caused by this rhizomorph-producing fungus.

In this connection it is of interest to note that Knobview, where the writer obtained his first cultures of the grape root-rot fungus, from which material he succeeded in rearing the fruiting bodies of *Clitocybe tabescens*, is but about 70 miles by air line from Bushberg, where Scribner (*46, p. 137*) reported the discovery of grape root rot in this country in 1887. The evidence at hand strongly indicates that the organism isolated by the writer, which causes a more or less serious root rot of grapevines in many sections of southern Missouri, was likewise the cause of the root rot reported 38 years ago, rather than *Armillaria mellea*, as is generally believed according to Piper and Fletcher (*39, p. 7*) and Hesler and Whetzel (*20, p. 97*).

On June 12, 1923, a series of cultures in Erlenmeyer flasks was begun with a view to observing the production of rhizomorphs and fruiting bodies where a large quantity of the substratum and a larger space for growth were provided. Six cultures were made in 500 c. c. flasks containing a quantity of small pieces of roots of a wild grapevine (*Vitis aestivalis* Michx.). Twelve other cultures were made in 150 c. c. flasks filled to a depth of three-quarters of an inch with agar. Six of these contained maltose agar, the other six raisin agar. One of each of these three sets of six flasks was inoculated with a pure culture of the Florida form causing root rot of eucalyptus, which was kindly furnished by Miss C. Audrey Richards, of the Madison branch of the Office of Investigations in Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture. The remaining five flasks in each of the three sets of six were inoculated with pure cultures of the grapevine root-rot fungus secured by the writer at Knobview, Mo.

All the cultures made a slow but steady growth after the lapse of a few days. The different cultures presented certain minor variations in the appearance of the superficial mycelium, but no greater difference appeared between the two root-rot fungi than occurred in any one group of the three different substrata. The cultures all exhibited the same general appearance in so far as the aerial mycelium was concerned. The marginal growth, as a rule, was rather sparse and downy-appressed. At the center of the cultures the mycelium developed into thickened compact masses which sometimes were quite nodular at first. In some instances the downy-appressed marginal growth was lacking, the feltlike mycelial growth being thickened and convex clear up to the margin. The mycelium was white at first, but soon turned to a dirty white, later changing to isabelline or light buff, and eventually becoming light tan in the older portions.

After the lapse of from ten days to two weeks it was noted that in all of the six cultures on raisin agar the agar had become strikingly discolored, becoming dark brown in advance of the mycelium, whereas such a discoloration did not occur in any of the cultures on maltose agar. When but a

[5] In this connection it is of interest to note that Totten (*53*) reported cultural studies of this fungus (as *Clitocybe caespitosa*), but made no mention of the production of fruiting bodies. A report of the writer's successful rearing of the sporophores of this fungus was given in the following: RHOADS, A. S. CLITOCYBE PARASITICA AS A CAUSE OF ROOT ROT OF GRAPEVINES. Paper presented at 17th Annual Meeting, Bot. Soc. Amer. (Mycol. Sect.), Cambridge, Mass., December, 1922. [Not published. Title in program, p. 13.] On the same program there also appeared an abstract of a paper by Miss C. Audrey Richards (*41*) in which report was made of the rearing of sporophores of the latter fungus isolated from a piece of rotted eucalyptus root sent from Florida. *Clitocybe monadelpha* is but one of the several synonyms of *Clitocybe tabescens*, the discussion of the history and nomenclature of which will be deferred until later. These joint reports constitute the first record of the development of sporophores of this fungus in artificial cultures.

week old the cultures of eucalyptus root rot, on both maltose and raisin agars, quickly developed a number of light-brown branching rhizomorphs with whitish tips. These extended downward and outward from the central portion of the mycelial growth. The appearance of these structures was somewhat varied, but perhaps can be described best as at first antlerlike, closely resembling the branches of certain Clavarias. In some cases the ends were pointed; in others they were blunt and flattened.

When the cultures of the eucalyptus root-rot fungus were 2 weeks old, it was noticed that in both those on maltose and on raisin agar the ends of some of the rhizomorphs had turned upward and had continued growth in this position until they reached slightly above the surface of the agar. That the upward growth of these submerged organs was the result of their own volition and that they were not deflected upward by the walls of the flasks were clearly evident. The exposed ends were whitish and at their junction with the surface of the agar there soon developed outward a downy-appressed mycelial growth. In this way the growth of the fungus might be started anew at several points on the surface of the agar in advance of the superficial mycelium developed outward from the original point of inoculation. After reaching the surface of the agar the rhizomorphs grew but little longer. They rarely protruded above the surface of the agar more than from one-sixteenth to one-eighth inch.

With the increased age of the cultures the rhizomorphs gradually acquired a darker coloring until they became dark brown. When about 4 weeks old, it was noted on viewing the cultures by transmitted light that there radiated from many of the rhizomorphs a dense but delicate growth of whitish mycelium having an opalescent appearance, like the growth of certain bacteria in stab cultures. So dense was this halo of radiating mycelial growth that its filamentous character could be distinguished only at the periphery, even with a hand lens. Beginning at the ends of the rhizomorphs, or practically so, this radiating halo of mycelium gradually became broader, proceeding away from the end until it had attained a length of as much as 5 mm., measured radially from the rhizomorphs. The appearance called to mind the root-hair development on a radish seedling, except for the fact that the radiating mycelial threads were much more minute and dense in comparison. Other rhizomorphs without this root hairlike development of delicate radiating mycelial filaments had numerous little white branches from 1 to 3 mm. long that developed out of the old brown rhizomorph at more or less of a right angle, like the aerial roots along the stem of the English ivy. Sometimes there would be one every millimeter or two, or a space of several millimeters might occur without them. In other cases these branches would develop in pairs or even in tufts of several, some of which were branched. Eventually these numerous, short, more or less radially disposed lateral branches of the rhizomorphs also became brown. This is truly a remarkable fungus that is well worth an intensive cultural study.

In a number of the cultures in this series, chiefly those of the Florida form, clusters of mushrooms made their appearance after a more or less definite period of time and attained a more or less perfect stage of development. The first evidence of sporophore formation was the development of a little group of hornlike processes which quickly differentiated into a cluster of embryonic mushrooms. The time at which fruiting began is based in the tables upon the appearance of the latter. In practically every case the clusters of fruiting bodies developed first from the central portion, or oldest part, of the culture. After one cluster of mushrooms had started its development at this point, from one to five others sometimes appeared, developing beside the first one, or at the periphery of the central mycelial mass, or at points farther out from the central mycelial mass, such as the ends of the rhizomorphs which had turned upward and reached the surface of the agar, or from the mycelial growth developed where the ends of the rhizomorphs appeared at the surface of the agar. As a rule, only the cluster of mushrooms first appearing attained full development, and only a few of the sporophores within this cluster attained any great size. In a few cases, however, a second, and even a third, cluster of mushrooms developed in more or less rapid succession. The caps were hemispherical at first, but quickly became more or less sharply centrally depressed or depressed-umbonate as they expanded to their full development. The young sporophores presented a considerable variation in color, ranging from a creamy white when very young to buff or fawn color as they became somewhat older. As they approached maturity the marginal portion of the pileus

became yellowish white to buff and the central part darker and often ornamented with brownish fibrils. The gills were pure white at first and distinctly decurrent. No evidence whatever of an annulus was seen. As a rule, about a week was required for the embryonic sporophores to develop to maturity and shed spores, after which they quickly became sodden and discolored. Occasionally, the development of the sporophores was checked, and they dried up before shedding spores.

The Missouri form causing the grapevine root rot behaved essentially the same in culture as did the eucalyptus form from Florida described in the four preceding paragraphs, differing only in that it gave a very meager and more delayed development of rhizomorphs as compared with the latter. The Missouri form likewise developed fruiting bodies in the cultures much more slowly and with much more difficulty than the Florida form. The development of these two forms of *Clitocybe tabescens* may be compared in Table I, which shows the length of time required for each to reach certain definite stages of development on three different substrata. Inasmuch as the writer left Missouri, the cultures were abandoned after the lapse of 110 days.

The fruiting bodies which the writer succeeded in developing from the cultures of the Missouri form in this series of cultures and in other miscellaneous ones were an exact counterpart of these developed by cultures of the Florida form, and the writer is convinced that these two forms of root rot represent simply two different strains of the same fungus, namely, *Clitocybe tabescens*. The more luxuriant development of rhizomorphs and stronger tendency to develop sporophores exhibited by the Florida form may possibly be an acquired environmental character.

On June 30, 1923, a series of cultures of the Florida form was started from basidiospores taken from a spore print cast on the side of the tube in which a group of mushrooms had developed in a former culture. This series was made with a view to determining the length of time required to run this fungus through its full life cycle from spore to spore and to compare this with the length of time required for mycelial transfers of the same form to produce fruiting bodies. Unfortunately, spores of the Missouri form were not available at this time, nor was the opportunity afforded the writer of collecting material in the field to furnish spores; so that no comparison can be made between the two forms of *Clitocybe tabescens* grown from the basidiospores.

The results of the experiment with the Florida form, presented in Table II, indicate that it makes no difference in the length of time required for this form to develop fruiting bodies,

TABLE I.—*Flask cultures for comparison of the Florida and Missouri strains of Clitocybe root rot*

| Culture medium and number of culture | Source of organism | Number of days required for— | | |
|---|---|---|---|---|
| | | Appearance of rhizomorphs | Differentiation of sporophores | Maturation of sporophores |
| Pieces of *Vitis aestivalis* root: | | | | |
| 1 | Eucalyptus from Florida | 22 | 44 | 50 |
| 2 | Grapevine from Missouri | 15 | | |
| 3 | do | 31 | 76 | 83 |
| 4 | do | 33 | | |
| 5 | do | 37 | | |
| 6 | do | 37 | | |
| Maltose agar: | | | | |
| 1 | Eucalyptus from Florida | 7 | 37 | 50 |
| 2 | Grapevine from Missouri | 22 | 75 | 86 |
| 3 | do | 35 | | |
| 4 | do | 35 | 77 | 87 |
| 5 | do | 37 | | |
| 6 | do | 37 | | |
| Raisin agar: | | | | |
| 1 | Eucalyptus from Florida | 7 | 36 | 42 |
| 2 | Grapevine from Missouri | 18 | | |
| 3 | do | 18 | | |
| 4 | do | 18 | | |
| 5 | do | 21 | | |
| 6 | do | 35 | | |

whether the cultures are started from freshly cast basidiospores or mycelial transfers. In all cases the basidiospores quickly gave rise to a sparse, downy-appressed mycelium, the central part of which developed into a tough, convex, light tan mycelial mass, the surface of which was very finely and densely nodular. The marginal growth about this initial mycelial mass was a sparse, downy-appressed fawn to tan-colored mycelium. In their subsequent growth and behavior these cultures of the Florida form started from basidiospores (pl. 5) agreed exactly with those of the same form started from mycelial transfers.

TABLE II.—*Growth of the Florida strain of Clitocybe root rot in cultures made from basidiospores*

| Culture medium and number of culture | Number of days required for— | | |
|---|---|---|---|
| | Appearance of rhizomorphs | Differentiation of sporophores | Maturation of sporophores |
| Raisin agar (three small test tubes): | | | |
| 1 | 7 | 31 | 37 |
| 2 | 7 | 31 | 37 |
| 3 | 8 | 32 | 38 |
| Maltose agar (three large test tubes): | | | |
| 1 | 7 | 31 | 38 |
| 2 | 8 | 31 | 38 |
| 3 | 9 | 31 | 38 |
| Maltose agar, (three 150 c. c. flasks): | | | |
| 1 | 8 | 33 | 38 |
| 2 | 9 | 34 | 41 |
| 3 | 9 | 36 | 43 |

## HISTORY, NOMENCLATURE, AND GEOGRAPHIC DISTRIBUTION OF FUNGUS

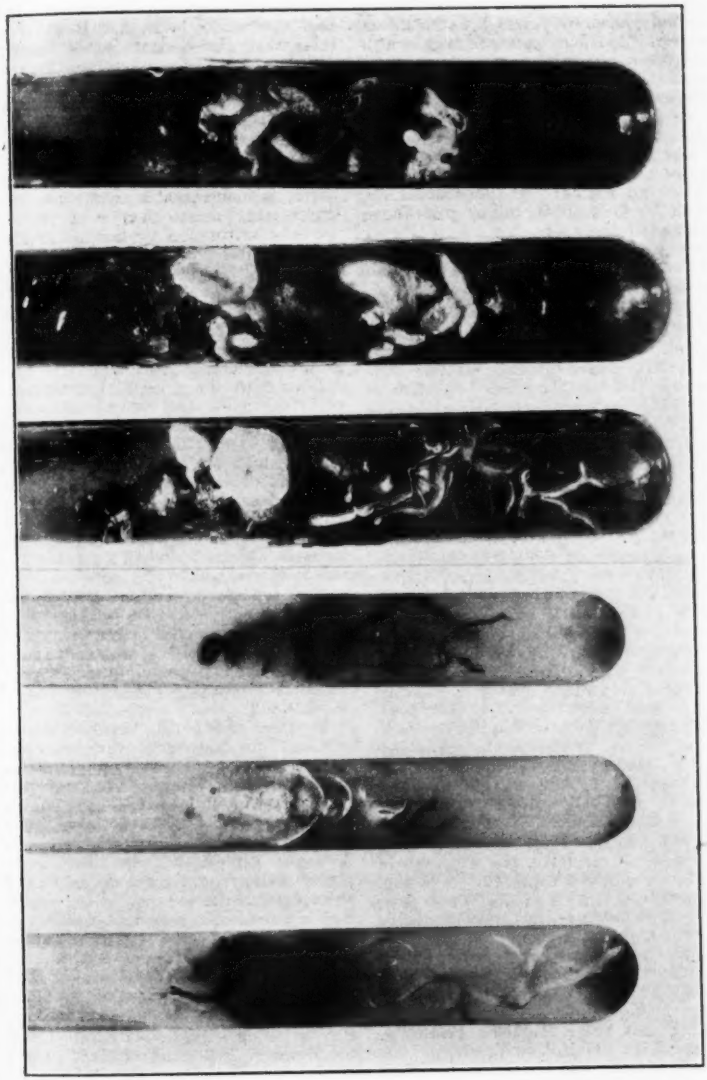
Early in the course of the investigation it became evident that although the species of Clitocybe causing the root rot of grapevines in Missouri was identical with Morgan's *Agaricus monadelphus* (*27, p. 69*), this was but one of several names that had been given to the same plant in the United States. Moreover, Bresadola's decision in 1900 (*6, p. 84–85*) that the plant which we have known best in the United States as *Clitocybe monadelpha* is the same as *Clitocybe tabescens* of Europe, which is accepted by practically all American mycologists who are familiar with this plant, adds still more to the multiplicity of names for it.

While various writers have from time to time given a partial list of the synonyms of this fungus, each has included but a small portion of the large total. The present writer, having made an extensive survey of the literature on this subject, has deemed it advisable and highly desirable, therefore, to review the history, nomenclature and geographic distribution of this fungus with the hope that its exact status and the relation of its numerous synonyms may be definitely settled and established.

*Clitocybe tabescens* (Scop.) Bres. is based upon Scopoli's description of *Agaricus tabescens* in 1772 (*45, p. 446*), Bresadola having decided that Scopoli's species represents the earliest authentic description of this much-named plant. Although the history of the plant in question appears perfectly clear since that date, the status of Scopoli's species and its relation to the species described by certain contemporaneous writers is somewhat of a mystery and probably will always remain so.

As a synonym of his *Agaricus tabescens*, Scopoli cites Haller's "Fungus siccior, pulvinatus, rufus, lamellis rarioribus," with the note "huic proximus," Scopoli's citation of the number of Haller's species, however, should have been 74 instead of 47. As synonyms of his species Haller (*19, p. 49, no. 74*) cites Micheli's "Fungus parvus, esculentus, odoratus, coriaceus, rufus, lamellis inter se longe distantibus" (*26, p. 148, no. 3*), with a question mark and the note, "Non repugnat," and Vaillant's "Fungus multiplex sordide carneus" (*55, p. 66, no. 36*), with the note, "Non recedit."

*Agaricus gymnopodius* illustrated by Bulliard (*7, pl. 601*) in 1780 and *A. socialis* described by De Candolle (9, *p. 48*) in 1815 are accepted by



Lower figure—Cultures of *Clitocybe tabescens* (Florida form causing root rot of Eucalyptus) developed on maltose agar 19 days after inoculation with basidiospores, showing the production of rhizomorphs. About natural size

Upper figure—Cultures of *Clitocybe tabescens* (Florida form causing root rot of Eucalyptus) developed on maltose agar 38 days after inoculation with basidiospores, showing the production of rhizomorphs and mature sporophores. About natural size

Bresadola (*6*) and others as synonyms of Scopoli's *A. tabescens*. *A. glomeratus* described and illustrated in 1824 by Pollini (*40, p. 679*), which, as he states, is an "*Agaricus gymnop.* aggregatus caespitosus saepius ramoso-connatus," etc., is not included as a synonym by Bresadola, although in Saccardo's Sylloge Fungorum (*43, p. 385*) it is included in the list of illustrations of *Clitocybe tabescens*, or *Collybia tabescens* as he calls it, following Fries. With allowances for poor drawing, Pollini's illustration might pass for a cluster of the sporophores of *Clitocybe tabescens* in which the bases of the stipes had anastomosed as a result of growth conditions.

Fries, who clearly had but little familarity with the plant or plants which we now know as *Clitocybe tabescens*, added greatly to the confusion. In his "Epicrisis" in 1836 to 1838 (*17*) he described, without having seen either of them, *Agaricus tabescens* under Tricholoma (*17, p. 33*) and *A. gymnopodius*, which he knew only from Bulliard's illustration, under Flammula (*17, p. 183*), assuming that the spores were colored because the gills were. As synonyms of *A. tabescens*, Fries cited Scopoli's description and illustrations by Battarra, Larber and Micheli, the latter being Micheli's Plate 74, Figure 2, on which Persoon based his *A. buxeus*. None of these illustrations cited by Fries bears the slightest resemblance to *Clitocybe tabescens* as understood by Bresadola. In a later work in 1874 (*18*) Fries exhibited no greater familiarity with these species, retaining *A. gymnodius* under Flammula (*18, p. 244*) and transferring *A. tabescens* to Collybia (*18, p. 111*), although uncertain as to whether or not the gills were decurrent and whether the spores were colorless or colored. His account of the latter species is especially confusing. After its description as No. 344 on page 111, in which he cites only Scopoli's description and *A. socialis* in the sense of De Candolle (*9*) and Seynes (*49*), he describes it again but differently in his appendix to Agaricus on page 319, again citing Scopoli's description but adding this time Micheli's Plate 74, Figure 2, on which Persoon (*37, p. 190*) based his *A. buxeus* in 1828. This illustration of Micheli represents his "Fungus pileolo desuper lacero," etc. (*26, p. 158*), which is a different species from that mentioned above as having been cited by Haller and upon which Scopoli based his *A. tabescens*. Since Fries does not cite the synonym which Scopoli gives in his description of *A. tabescens*, it appears that he disagrees with Scopoli's use of this as a basis of his species, for on page 704, under addenda, he says: "P. 319. *A. tabescens*. Hic diversus est a supra n. 344 citato. Utri synonymon Scopolii sit referendum, dubium." Even if this be so, the writer can not see any justification for Fries's making two kinds of *A. tabescens* out of Scopoli's brief description.

From the foregoing it is evident that there is considerable confusion and uncertainty as to just what species authors writing before Scopoli in 1772 considered as synonyms of his *Agaricus tabescens*. However, it is virtually of no taxonomic value, and is even futile from the standpoint of accuracy to attempt to trace this species back to the days before the existence of binomial nomenclature.

Owing to the universal acceptance of Fries's work, the views held by him have been perpetuated in subsequent writings. Thus, in Saccardo's "Sylloge Fungorum" (*43, p. 385*), which follows Fries, Micheli's figure of his "Fungus pileolo desuper lacero, etc.," is cited as an illustration of *Collybia tabescens*, as Saccardo calls it, although in the writer's opinion Micheli's figure does not even resemble the species. Persoon's *Agaricus buxeus* (*37*), which was based upon this illustrated species of Micheli's, is accepted as a synonym of *Agaricus* (*Collybia*) *tabescens* by Winter (*59, p. 852*), and doubtfully so in Saccardo's "Sylloge Fungorum" (*42, p. 12*).

In the middle of the nineteenth century the history of the American plant which we know to-day as *Clitocybe tabescens* began. It was collected first in Ohio by Lea, who sent it to Berkeley. In 1847 the latter published a description of it (*1*), calling it *Lentinus caespitosus*. In 1868, supplemented by additional specimens of the plant collected by Ravenel in South Carolina, it was renamed *Agaricus* (*Pleurotus*) *caespitosus* by Berkeley and Curtis (*2*), apparently on the advice of the latter, who stated that "this is certainly an agaric."

One year previous to this, however, Curtis (*11, p. 85*), in his catalogue of the indigenous and naturalized plants of North Carolina, lists "*Clitocybe caespitosus*, M. A. C." under Agaricus. Although Totten (*53*), on the strength of Curtis' citation, used the name *Clitocybe cespitosa* for the plant we now know as *Clitocybe tabescens*, Curtis's name must be regarded as a *nomen nudum*. Neither *Clitocybe caespitosa* Peck (*31, p. 61*) nor *Clitocybe caespitosa*

Pat. (*30, p. 248*) should be confused with Curtis's name, for they represent entirely different species.

In 1883, Schulzer von Muggenburg (*44, p. 256*) described as a new species a plant occurring in Hungary, Poland, and Transylvania, which he had had under observation for several years and regarded as an exannulate form of *Agaricus* (*Armillaria*) *melleus*, naming it *Agaricus* (*Collybia*) *inarmillatus*. This was regarded as a synonym of *Clitocybe tabescens* by Bresadola (*6*).

In the same year Morgan (*27*) described from Ohio a plant which closely resembled *Agaricus* (*Armillaria*) *melleus*, but which lacked an annulus. This he called *Agaricus monadelphus*, placing it under Clitocybe.

In 1892 Peck (*32, p. 180–181*) published Miss Banning's description of *Clitocybe aquatica*, based upon specimens from Maryland. A year later Peck (*33, p. 134*) under his description of *Armillaria mellea*, mentions the receipt from Brooklyn, N. Y., and Washington, D. C., of a densely caespitose, slender-stemmed form with no annulus, which he called var. *exannulata*. The following remark by him is of interest: "It is scarcely distinguishable from *Clitocybe aquatica* Banning, and *Clitocybe monadelpha* Morg., which, I suspect, will yet have to be referred to this species. According to Quelet, *Clitocybe socialis DC.*, and *Agaricus gymnopodius* Bull. also belong here."

In 1895 Peck (*34, p. 265*) described *Armillaria mellea* var. *exannulata* in greater detail. In 1898 he reported for the first time the occurrence of *Clitocybe monadelpha* in New York (*35, p. 284*) and described and illustrated the species (*35, p. 302–303, pl. 51, figs. 1 to 5*). In his report on the edible fungi of New York in 1900, Peck (*36*) reproduced the same description of *Clitocybe monadelpha*, the illustrations being changed somewhat.

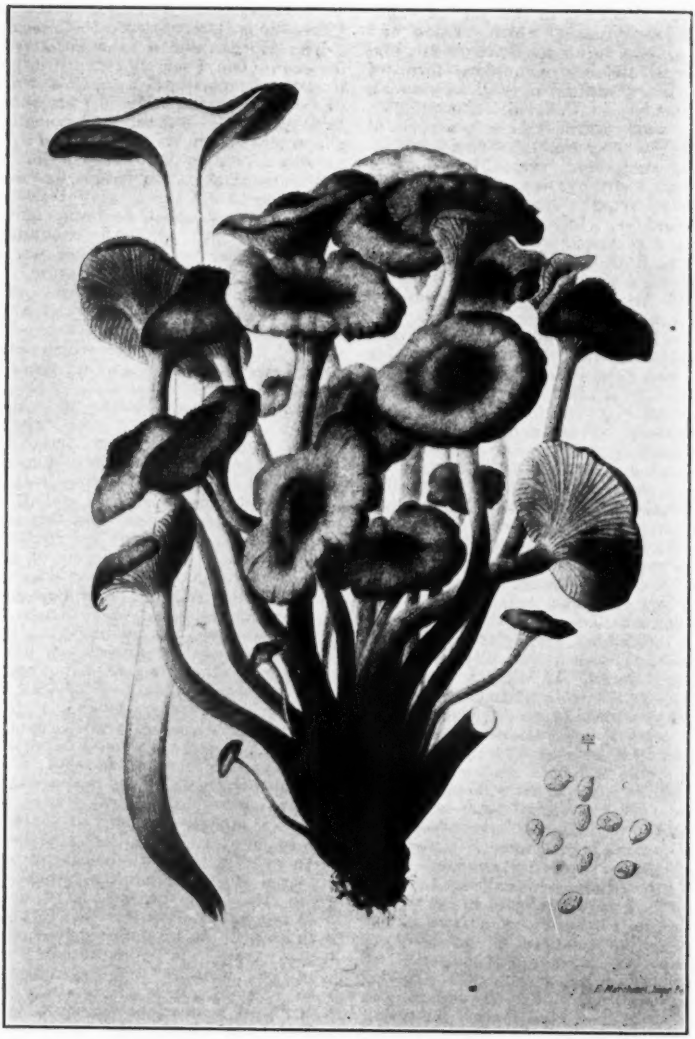
In 1900 appeared Bresadola's work (*6*), in which he pointed out that the plant we have known best in the United States as *Clitocybe monadelpha* is but a synonym of *Clitocybe tabescens* of Europe. It is generally conceded by most American mycologists familiar with this plant that Bresadola was correct in considering the American plant to be the same as the European. Boudier's excellent illustration, appearing as Plate 61 (51a) in his "Icones Mycologicae ou Iconographie des Champignons de France" and reproduced with slight modification as Plate 197 of Bresadola's work, is reproduced here as Plate 6. It is typical of our plants.

In his paper on a rhizomorphic root rot of fruit trees in Oklahoma caused by a caespitose species of Clitocybe, Wilcox (*58*) states that this species of Clitocybe is closely related to *C. monadelpha* Morgan and to *Armillaria mellea exannulata* Peck, but he describes it as a new species, *C. parasitica*, since he believed that it differed from these two species in certain morphological characters and in its parasitic habit of growth.

Unfortunately, the writer has not been able to see the type of Wilcox's species. Specimens do not occur either at the Missouri Botanical Garden, where he spent a few weeks in examination of the literature, or at the Oklahoma Agricultural and Mechanical College, where, if any were deposited, they probably were destroyed in the 1914 fire which consumed the herbarium and all records pertaining thereto.

Almost the only points in which Wilcox's description of his species differs from Morgan's are these: In Wilcox's plant the pileus is roughened with small scales from the first instead of being at first glabrous and then scaly; the stipes are never twisted, and it "is always parasitic in habit." According to the observations of the writer on the plants in both Missouri and Florida, Wilcox's morphological distinctions are based on rather variable characters. It is obvious that fungi never can be stereotyped creations and that mycologists must allow a certain amount of latitude in the species concept, which, in this case at least, is but arbitrarily defined. Citing a statement in one of Morgan's letters to him, written in 1901, that *C. monadelpha* is never a parasite, Wilcox reports (*58, p. 18*) that "our species is always parasitic in habit," and at the bottom of the same page further states that "the species grows in dense clusters from the crowns of living trees and the stumps of dead trees but is strictly a parasitic form in both cases." In his summary on page 22 he says: "The same fungus is a common parasitic and saprophytic form on four species of oak in Oklahoma." It is obvious, therefore, that neither the distinctions made by Wilcox in the morphological characters nor those in the habit of growth of these two fungi hold good.

Wilcox regarded the Oklahoma form as distinct from Peck's *Armillaria mella exannulata* because the plants



Reproduction of Boudier's illustration of *Clitocybe tabescens* given as Plate 61 (51a) in his "Icones Mycologicae ou Iconographie des Champignons de France," (5). Reduced to about ⅓ size of original plate

were larger and the caps not smooth, as Peck mentions in one place for his species, and because it never exhibited any evidence of an annulus even in the young stages, Peck's plant having occasionally had an evanescent annulus. These points of difference again clearly are variable characters and the writer is of the opinion that both plants should be included as synonyms of *Clitocybe tabescens* in the sense of Bresadola.

Murrill, in his monograph of the Agaricaceae of tropical North America (*28*) and in his monograph of the genera Clitocybe and Monadelphus in the North American Flora (*29, p. 420–421*), retains in Clitocybe the nonwood-loving caespitose species of Clitocybe and restricts the genus Monadelphus [6] founded by Earle (*15, p. 432*) to receive the caespitose species of Clitocybe, to include only those that are wood-loving.[7] Murrill makes no mention of *Clitocybe tabescens*, and Bresadola's determination that the plant we have known best in the United States as *Clitocybe monadelpha* is but a synonym of this species appears to have been overlooked entirely by him. The plant which is the subject of this paper is treated as *Monadelphus caespitosus*, based upon Berkeley's *Lentinus caespitosus*. Morgan's *Agaricus monadelphus*, Miss Banning's *Clitocybe aquatica* published by Peck, Peck's *Armillaria mellea exannulata*, and Wilcox's *Clitocybe parasitica* are all given as synonyms, although the latter is prefaced with a question mark.

Various other synonyms of *Clitocybe tabescens* not discussed here result from the raising of the older subgenera to full generic rank or the transfer of a species to various other genera. Taxonomic notes of historical interest are given on this species by Lloyd (*23, 24*). The treatment of this species by Kauffman (*22, p. 723*) and by Coker and Beardsley (*10, p. 106–107*) is also of interest.

There follows a chronological list of all the synonyms of *Clitocybe tabescens* in so far as they are known to the writer, those prefaced with a question mark being doubtful. Following this is a chronological list of all the illustrations of this species as far as they are known to the writer and believed to be correct, those prefaced with a question mark being doubtful.

SYNONOMY OF CLITOCYBE TABESCENS (SCOP.) BRES.

*Agaricus tabescens* Scop., Fl. Carn. ed. 2. 2: 446. 1772.

*Agaricus gymnopodius* Bull., Herb. Fr., Pl. 601, Fig. 1. 1793.

*Agaricus socialis* DC., Fl. Fr. 6: 48. 1815. Not *A. socialis* Fr., Ic. Hymen. 1: Pl. 49, Fig. 2. 1871; Hymen. Eur., p. 83. 1874.

?*Agaricus glomeratus* Pollini, Fl. Ver. 3: 679. 1824.

*Lentinus caespitosus* Berk., Lond. Jour. Bot. 6: 317. 1847.

*Agaricus* (*Pleurotus*) *caespitosus* Berk. & Curt., Jour. Linn. Soc. 10: 287. 1868.

*Flammula gymnopodius* (Bull.) Quél., Champ. Jura Vosg. 2: 346. 1873.

*Clitocybe gymnopodia* (Bull.) Gill., Champ. Fr., p. 162. 1874.

*Agaricus* (*Collybia*) *inarmillatus* Schulzer, Oesterr. Bot. Ztschr. 33: 256. 1883.

*Agaricus monadelphus* Morgan, Jour. Cinc. Soc. Nat. Hist. 6: 69. 1883.

*Clitocybe monadelpha* (Morgan) Sacc., Syll. Fung. 5: 164. 1887.

*Collybia tabescens* (Scop.) Sacc., Syll. Fung. 5: 206. 1887.

*Pleurotus caespitosus* (Berk. and Curt.) Sacc., Syll. Fung. 5: 352. 1887.

*Clitocybe aquatica* Banning and Peck, Ann. Rpt. N. Y. State Mus. 44: 180. 1892.

*Armillaria mellea exannulata* Peck, Ann. Rpt. N. Y. State Mus. 46: 134. 1893.

*Clitocybe tabescens* (Scop.) Bres., Fungi Trid. 2: 84. 1900.

*Clitocybe parasitica* Wilcox, Okla. Agr. Exp. Sta. Bul. 49: 18. 1901.

*Monadelphus caespitosus* (Berk.) Murrill, Mycologia 3: 192. 1911.

ILLUSTRATIONS OF CLITOCYBE TABESCENS (SCOP.) BRES.

Bulliard, Herb. Fr., Pl. 601, Fig. 1. 1793. (As *Agaricus gymnopodius.*)

?Pollini, Fl. Ver. 3: Pl. 2, Fig. 6. 1824. (As *Agaricus glomeratus.*)

Sicard, Hist. Nat. Champ., Pl. 31, Fig. 163. 1883. (As *Agaricus gymnopodius.*)

Morgan, Jour. Cinc. Soc. Nat. Hist. 6: Pl. 4. 1883. (As *Agaricus monadelphus*); also reproduced by Wilcox, Okla. Agr. Exp. Sta. Bul. 49: Pl. 6. 1901.

[6] It is of interest to note that this genus was given the variant spelling of "Monodelphus" by Earle in his generic description on page 432. This appears to be a typographic error, however, for it was spelled the usual way in his key on page 403.

[7] This is truly a superficial distinction for placing a group of species with the same general character in different genera. Even when collected carefully, it is not always clear whether these plants are growing from the ground alone or from buried wood, and, in case this point is not noted by the collector, the difficulty of making a correct determination of the species by these keys is increased.

Banning, Fungi of Maryland (MS. in Herbarium N. Y. State Mus.), Pl. 46. 1889. (As *Clitocybe aquatica.*)

Peck, Ann. Rpt. N. Y. State Mus. 51: Pl. 51, Figs. 1 to 5. 1898. (As *Clitocybe monadelpha.*)

Peck, Mem. N. Y. State Mus. 3: Pl. 46, Figs. 7 to 12. 1900. (As *Clitocybe monadelpha.*)

McIlvaine, Amer. Fungi, Pl. 27. 1900. (As *Clitocybe monadelpha.*) In Plate 16, Fig. 2, a poor illustration is given of Peck's *Armillaria mellea exannulata,* which is reproduced by Wilcox, Okla. Agr. Exp. Sta. Bul. 49: Pl. 4, Fig. 4. 1901.

Bresadola, Fungi Trid. 2: Pl. 197. (As *Clitocybe tabescens.*)

Wilcox, Okla. Agr. Exp. Sta. Bul. 49: Pls. 1 to 3, 5, 7 to 9; Figs. 10, 11, 14 to 16, 19. 1901. (As *Clitocybe parasitica.*)

Boudier, Ic. Myc. 1: Pl. 61 (51a). 1905–10. (As *Clitocybe tabescens.*)

Hard, Mushr., Pl. 12 (fig. 75). 1908. (As *Clitocybe monadelpha.*)

Duggar, Fungous Diseases of Plants, Fig. 234. 1909. (As *Clitocybe parasitica.*)

Rolland, Atlas, Pl. 25. 1910. (As *Clitocybe gymnopodia.*)

Coker and Beardslee, Jour. Elisha Mitchell Sci. Soc. 38: Pl. 12; 33, Fig. 7. 1922. (As *Clitocybe tabescens.*)

On his map in Plate 11, Wilcox (*58*) shows the localities in which occurs the rhizomorphic root rot described by him as found in Texas, Oklahoma, Missouri, Illinois, Indiana, Ohio, and Georgia, and adds that California and Oregon should have been marked as States from which the same disease had been reported. The rhizomorphic root rot reported by Wilcox from the two latter States, and assumed by him to be due to *Clitocybe parasitica,* was in all probability caused by *Armillaria mellea,* which the writer knows from personal observation is of widespread occurrence on the Pacific coast. So far as the writer's information extends, there never has been an authentic report of Clitocybe root rot occurring on the Pacific coast or in the Pacific Northwest.

From the reports of Peck we know that the species of Clitocybe that we now call *C. tabescens* occurs as far north as New York on the Atlantic coast. Kauffman (*22*) reported the occurrence of *Clitocybe monadelpha* in Michigan, and Fawcett (*16, p. lxvi*) reported *Clitocybe parasitica* as causing a root rot of peach in Florida. The cultures of Clitocybe root rot mentioned above as furnished by Miss Richards were also from Florida, having been isolated from a rotted eucalyptus root. Since coming to Florida in 1923 the writer has collected specimens of *Clitocybe tabescens* growing at the bases of stumps of *Washingtonia robusta* and *Ilex opaca* at Gainesville, and at the base of a living oak tree at Cocoa. He has also observed two instances of this fungus causing a root rot of guava (*Psidium guajava*), one at Cocoa and the other near Courtenay on Merritt's Island.[8]

Murrill (*28, p. 193*) states that the species also occurs in Mexico and British Honduras. According to the taxonomic literature previously cited in this paper, the plant has a wide distribution in Europe. Nowhere in the European literature, however, has the writer seen any mention of its causing a rhizomorphic root rot of woody plants. In fact, even the cultural characters of the European form appear to be unknown.

In the United States the range of *Clitocybe tabescens,* which extends from New York south to Florida and west to Michigan, Kansas, and Texas, overlaps to a large extent the range of the closely related *Armillaria mellea,* which likewise causes a rhizomorphic root rot. Except for a few middle-eastern States, however, root rot caused by *Clitocybe tabescens* has been reported only from the southern part of the United States, where it is of most widespread occurrence.

## RELATION OF CLITOCYBE ROOT ROT TO TIMBERED LANDS AND DRAINAGE

The root rot of grapevines and fruit trees caused by *Clitocybe tabescens* is associated with lands which were formerly covered with hardwood timber, especially oak. This disease appears to be practically unknown in strictly prairie soils and in old land, except at the margins in close proximity to timbered land. Men who have had no experience with the disease on old land have found that it quickly made

[8] Thus far the writer has not found grapevines attacked by Clitocybe root rot in Florida, but has had virtually no opportunity to investigate this problem since coming to this State. In a case of root rot observed on a single Herbemont vine at Bartow the fungus associated with the root rot did not appear to be Clitocybe nor did the one isolated prove to be a rhizomorph-producing fungus. Although cultures have been maintained for several months, neither spores nor fruiting-bodies have been secured.

its appearance upon extending their plantings into ground more recently cleared.

In all cases where the writer has had the opportunity to observe Clitocybe root rot, both of grapevines and fruit trees in Missouri, it has occurred in the low and poorly drained parts of vineyards and orchards where water is liable to stand in the soil, although it may also occur in soils underlaid with an impervious subsoil or hardpan, where natural drainage is poor. In Florida, however, Clitocybe root rot has been observed in sandy soils that appeared to be well drained. The relation of Clitocybe root rot to inadequate drainage was illustrated in very striking fashion in a vineyard about 18 years old at Neosho. This was planted in Moore's Early grapes on one side and Concord on the other. The land was practically level. Immediately adjoining one end of the vineyard was a sorghum field in which, close to the end of the vineyard, lay a large, slightly depressed area where water stood for long periods during the early summer. Even the sorghum in this vicinity had made but poor growth and appeared stunted and pallid. The grapevines at this end of the vineyard were weak and unthrifty. Large numbers had died out and been removed (pl. 7, upper figure), and still others were dying or were practically dead. Several of the dying vines were dug up and found to have the mycelium characteristic of Clitocybe root rot. Clusters of the mushrooms had even developed from the root crowns in some cases. In addition, it was determined by the cutting method previously referred to that many of the living vines which appeared to be at least moderately healthy were infected by the fungus. At the other end of the vineyard, which was 520 feet in length and contained but 52 vines, there was a full stand growing vigorously (pl. 7, lower figure).

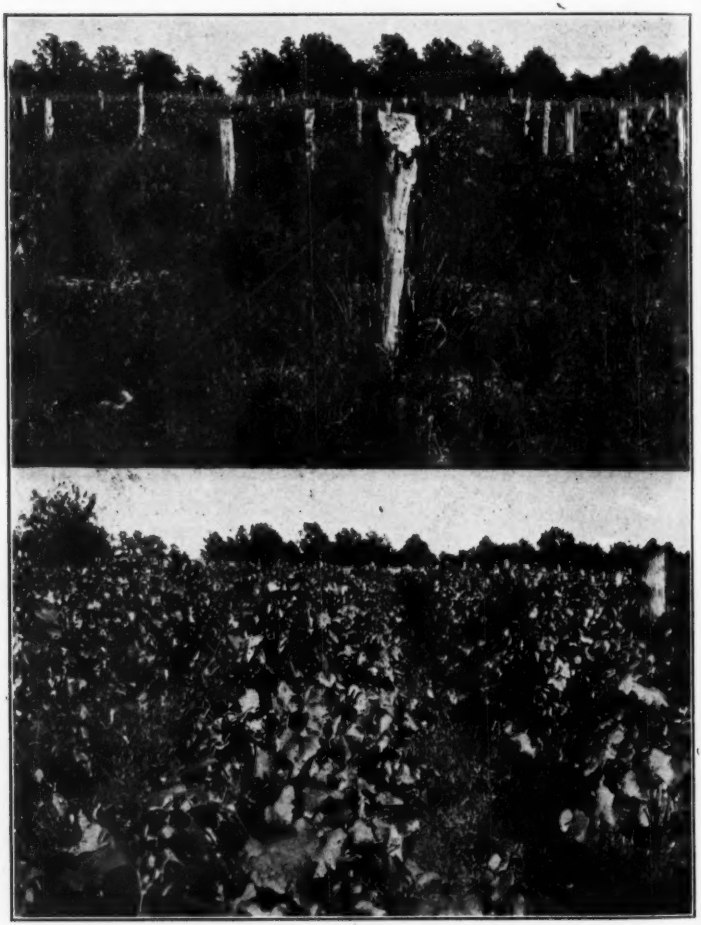
The losses occasioned to vineyards by Clitocybe root rot vary greatly. In mild cases perhaps just a few vines will die from year to year, but in severer cases they may die quite rapidly until within a few years parts of the vineyard may be heavily depleted, as in the case just cited.

## CONTROL OF CLITOCYBE ROOT ROT OF GRAPEVINES

A little attention directed to preventive measures will obviate the necessity of applying remedial measures in combating root rot which at best are but palliatives. In selecting a site for vineyard planting, newly cleared land, especially in a region of oak forests, should be avoided, since the fungus may live in the soil as a saprophyte on deadwood, such as stumps and roots. The character of the disease suggests the need for thorough preparation of cleared lands, including the removal of all roots, and cultivation for at least three years in other crops before setting out to grapevines or fruit trees. In any case, land which promises poor natural drainage should be avoided unless artificial drainage can be assured at a reasonable cost. Care should also be taken to prevent barking or otherwise injuring the roots of vines when cultivating the vineyard, since such injuries favor infection by root-rotting fungi. Control of the grapevine root borer is also essential for the same reason.

After Clitocybe root rot appears in a vineyard the vines that have been seriously attacked can not be saved; however, measures can be taken to check the spread of the disease and to save those recently attacked. Inasmuch as an excess of moisture in the soil, either as a result of a natural depression or being underlaid by an impervious substratum, is one of the chief conditions favoring the development of Clitocybe root rot, adequate drainage is, and will always be, the most valuable preventive and corrective measure. Unless this precaution is taken all others are useless, for vineyards that have become infected with this root-rot fungus are, unless drained, always subject to attack by it. The low or otherwise poorly drained places in the vineyard where water is liable to stand in the soil should receive most careful attention in this respect, for it is at these points that root rot is most likely to occur. As soon as the root rot is discovered in a vineyard all vines that have been killed by it and all vines badly diseased, that is, those whose scanty growth shows that their root system is seriously attacked, should be removed. These should be excavated carefully, care being taken to remove all the roots, which should be burned. Then, if the hole is left exposed to the air and sun for a few weeks or disinfection of the soil is accomplished by the use of chemicals, and the drainage of the soil has been assured, it should be safe to replant.

In European practice a number of chemicals have been applied to the soil to destroy the mycelium and spores of *Dematophora necatrix* and other root-rot fungi in the soil or to destroy the fungus on recently infected vines with-



(Above).—Depletion of Concord vines by Clitocybe root rot in poorly drained end of vineyard. These vines continue to die out from year to year

(Below).—Full stand of thrifty Concord vines in well-drained end of vineyard shown above, the undrained end being in the background. The rows are 520 feet in length and contain only 52 vines. The transition between the sickly vines at one end and the vigorous ones at the other was rather abrupt

out injuring the vine itself. Bonjour (*4*) recommends the use of a 7 per cent solution of green vitriol (ferrous sulphate). Beniling and Behaix likewise recommend green vitriol against rot of vine roots where carbon bisulphide and sodium fluoride had no action. Dufour (*12*), however, who has conducted experiments to control pourridié of the grapevine for a number of years, tested comparatively the action of green vitriol and blue vitriol on the causal fungus (*Dematophora necatrix*) and found the latter much superior. He recommends a 3 per cent solution of blue vitriol (bluestone or copper sulphate) as a soil disinfectant. This is applied by excavating a small basin around the trunk of the vine and pouring into it a gallon or so of the solution, the exact quantity being determined by the nature of the soil and the supposed extent of the root system. Before planting new vines, 1 to 2 quarts of the same solution are poured into the holes. Vines adjoining those known to be attacked are also laid bare in the spring and watered freely with the solution; 3 to 5 ounces of granulated bluestone per stock may likewise be spread over the ground. The result is not always completely visible the first year, and it is sometimes necessary to repeat the treatment for two years in succession, in which case the results obtained are claimed to be very positive.

Dufour likewise tried ammonium sulphide, hyposulphite of soda and sulphite of lime against *Dematophora necatrix* without securing any promising results. Potassium sulphocarbonate, which Dufour and Mouillefert tried, also proved disappointing. Narbonne experimented with sulphur and advises laying bare the less badly attacked stocks as deep as possible and dusting the roots abundantly with sulphur, repeating the dusting several times before the stocks are covered again.

Carbon bisulphide has been tested extensively by numerous investigators for the control of insects and root rot in vineyards. Dufour made applications at the rate of 200 gm. per square meter, after having removed the diseased roots. According to this eminent investigator, carbon bisulphide destroys the mycelium of the fungus to a large extent. Blunno (*3*), experimenting later in Australia, states that carbon bisulphide applied at the rate of 1 ounce per vine, divided into five partial injections made within 6 to 8 inches from the stem, was effective in killing the mycelium living externally, that is, during the first stage of infection. Such a dose, however, was not sufficient to destroy the old foci of infection represented by the roots of the once existing trees, and repeated and stronger doses endangered the vines. According to the same author, ferrous sulphate applied each winter for three successive years at the rate of 8, 16, and 16 ounces, respectively, to each vine was sufficient to restore all but two vines in which the root rot yielded to a further and slightly modified treatment consisting of daubing the infected part of the stem with a solution made up of 10 per cent by weight of sulphuric acid and 50 per cent of ferrous sulphate. The ferrous sulphate was used in preference to copper sulphate because it was much cheaper. It was applied in the usual way by digging a hole around the stem 6 inches deep with a radius of 12 inches, at the bottom of which the chemical was scattered and the soil then replaced. This salt was thought to act not only as a fungicide but also as a vigorous stimulant to growth.

Szigethi-Gyula (*52*) states that experiments in a vineyard located in a rather moist situation showed that the application of lime at the rate of 2 kg., or 10 liters of milk of lime, to a vine was efficient in preventing pourridié.

Inasmuch as the mycelium of the root-rot fungi is often more resistant to toxic substances than the host plant, considerable experimentation will be necessary before there will be found for the control of root-rot fungi an efficient and satisfactory fungicide that can be recommended for general use with safety to the vine. It seems to the writer that paradichlorobenzene, which has proved so successful in the control of the peach-tree borer, would be well worth a trial for the control of root-rot fungi. So far as is known, this chemical is not especially injurious to grapevines. The above-mentioned fungicides are not recommended for general use, but are merely suggested for those who may wish to experiment in a small way with them.

The comparatively simple method of aeration and exposure to sunlight also has shown encouraging results in the control of root rot, both on grapevines in Europe and on fruit trees in this country. In this method the dirt is carefully excavated from about the root crown and larger roots and these are left exposed to the sun during the remainder of the summer, either with or without the application of a fungicidal wash. The soil is replaced before the advent of winter.

SUMMARY

A mushroom root rot of grapevines, of rather common occurrence in various localities in the Ozark section of Missouri, is reported and described in detail. The organism isolated from the roots of the diseased vines proved to be a very slow-growing but distinctive one, developing characteristic submerged rhizomorphs in the cultures. Fruiting bodies, while not associated with the diseased vines first studied in the field, were subsequently developed in some of the cultures. But a few days before the first fruiting bodies were secured in the cultures a species of Clitocybe was observed fruiting abundantly at the bases of rotted grapevines in a vineyard in another section of the State and in an oak forest a few miles distant. Cultures of the fungus isolated from the roots of these vines yielded a rhizomorph-producing organism identical with the one isolated from the first material studied. Although no opportunity of reproducing this root-rot disease by inoculations with pure cultures of the fungus isolated from infected vines has been afforded, it is believed that beyond all doubt the disease in question is caused by this rhizomorph-producing organism.

The fungus which is believed to be the causal organism is a species of Clitocybe described by various writers in this country under the names *C. monadelpha*, *C. aquatica*, *Armillaria mellea exannulata*, and *C. parasitica*, all of which are clearly different names for the same plant and identical with *Clitocybe tabescens* of Europe. A detailed discussion is given of the history, nomenclature, and geographic distribution of this fungus, together with complete lists of the synonyms and illustrations believed to be authentic.

A detailed description is given of the characteristics and behavior of the Clitocybe root-rot fungus in pure cultures and a comparison is made between the form isolated from grapevine roots in Missouri and one secured from a eucalyptus root from Florida. Although the writer's cultures of the Missouri form of Clitocybe root rot were generally much slower in their development and in the production of fruiting bodies than the Florida form, the two agreed so closely in their morphological characters that he is of the opinion that these two forms represent but two rather widely differing strains of the same fungus.

Although *Clitocybe tabescens* in the United States is known to occur from New York south to Florida and west to Michigan, Kansas, and Texas, cases of root rot caused by this fungus, with the exception of those in a few middle eastern States, have been reported only from the southern part of the country, where it appears to be of most widespread occurrence. The losses occasioned to vineyards in Missouri by Clitocybe root rot, which appears to be a comparatively slow-working disease, vary from the death of a few vines each year in cases of mild attack to such rapid destruction that within a few years parts of the vineyard may be heavily depleted.

Clitocybe root rot is a disease associated with lands which formerly were covered by hardwood timber, especially oak. The disease appears to be practically unknown in strictly prairie soils and in old land, except at the margins in close proximity to timbered lands. It has been found to attack grapevines and fruit trees chiefly in places where the soil is poorly drained, either as the result of natural depressions in contour or in places underlaid with a more or less impervious soil or hardpan.

Attention directed in the selection of vineyard sites to the thorough preparation of newly cleared timberlands, adequate drainage of any spots in need of it, and prevention of barking or other injury of the vine roots when cultivating the vineyard, will prove valuable measures in preventing attack by Clitocybe root rot. In case the disease is observed in vineyards already established, adequate drainage, which is and will always remain the most valuable remedial measure, should be assured, and the spread of the disease may then be checked by the prompt removal of all badly diseased and dead vines, the chemical treatment of adjacent vines, and the disinfection of the soil before replanting. The various methods for the destruction of the fungus on newly infected vines and in the soil are briefly reviewed and the need for further experimentation is pointed out.

Comparatively little study has been made of the fungi causing root rot of grapevines in this country. Although cases of this disease have been reported in various sections since its discovery in Missouri in 1887, it has, with few exceptions, been attributed to *Armillaria mellea* and *Dematophora necatrix*, as a rule on assumption, however, rather than from definite knowledge.

The present investigation affords very definite evidence that Clitocybe root rot, which hitherto has been certainly known to attack only fruit, forest, and shade trees, is responsible for a considerable quantity of root rot of grapevines, at least in Missouri, and probably also in other southern States. The isolation of this fungus by the writer from the roots of recently killed vines but 70 miles from the point where root rot of the grapevine was first discovered in this country indicates that *Clitocybe tabescens* was the cause of the case first reported rather than *Armillaria mellea*, as is generally believed.

## LITERATURE CITED


(1) BERKELEY, M. J.
1847. DECADES OF FUNGI. DEC. XII–XIV. OHIO FUNGI. Lond. Jour. Bot. 6: 312–326.

(2) ——— and CURTIS, M. A.
1868. FUNGI CUBENSES (HYMENOMYCETES). Jour. Linn. Soc. Bot. 10: 280–392.

(3) BLUNNO, M.
1911. ON THE PROPORTION OF FERROUS SULPHATE USED AGAINST THE "WHITE ROT" OF GRAPE VINES. Rpt. Austral. Assoc. Adv. Sci. 13: 562–566.

(4) BONJOUR, J.
1891. MANUEL PRATIQUE DU VIGNERON. Avec figures, chez Eugène Vodoz, à Vevey. [Not seen. Commented on by "S. B." in Chron. Agr., Vit. et Forest Vaud. 4: 272. 1891.]

(5) BOUDIER, E.
1905–10. ICONES MYCOLOGICAE, OU ICONOGRAPHIE DES CHAMPIGNONS DE FRANCE. t. 1, illus. Paris.

(6) BRESADOLA, G.
1900. FUNGI TRIDENTINI NOVI, VEL NONDUM DELINEATI, DESCRIPTI, ET ICONIBUS ILLUSTRATI. v. 2, illus. Tridenti.

(7) BULLIARD, P.
1780. HERBIER DE LA FRANCE. 602 col. pl. Paris.

(8) BUTLER, O.
1905. OBSERVATIONS ON SOME VINE DISEASES IN SONOMA COUNTY, CALIFORNIA. Calif. Agr. Exp. Sta. Bul. 168, 29 p., illus.

(9) CANDOLLE, AUG. DE
1815. FLORE FRANÇAISE. t. 5 (v. 6). Paris.

(10) COKER, W. C., and BEARDSLEY, H. C.
1922. THE LACCARIAS AND CLITOCYBES OF NORTH CAROLINA. Jour. Elisha Mitchell Sci. Soc. 38: 98–126, illus.

(11) CURTIS, M. A
1867. BOTANY; CONTAINING A CATALOGUE OF THE INDIGENOUS AND NATURALIZED PLANTS OF THE STATE. Geol. and Nat. Hist. Surv. N. C., v. 2, pt. 3, 156 p.

(12) DUFOUR, J.
1894. NOTE SUR L'EMPLOY DU VITRIOL BLEU CONTRE LE POURRIDIÉ. Rev. Internat. Vit. et Oenol. 1: 75–77

(13) DUGGAR, B. M.
[1909]. FUNGOUS DISEASES OF PLANTS, WITH CHAPTERS ON PHYSIOLOGY, CULTURE METHODS AND TECHNIQUE. 508 p., illus. Boston, New York [etc.].

(14) EARLE, F. S., and AUSTIN, C. F.
1900. GRAPES. Ala. Agr. Exp. Sta. Bul. 110, p. 55–91, illus.

(15) ———
1909. THE GENERA OF NORTH AMERICAN GILL FUNGI. N. Y. Bot. Gard. Bul. 5: 373–451.

(16) FAWCETT, H. S.
1912. REPORT OF THE PLANT PATHOLOGIST. Fla. Agr. Exp. Sta. Ann. Rpt. 1911: lviii–lxvii.

(17) FRIES, E. M.
1836–38. EPICRISIS SYSTEMATIS MYCOLOGICI, SEU SYNOPSIS HYMENOMYCETUM. 610 p., illus. Upsaliae.

(18) FRIES, E. M.
1874. HYMENOMYCETES EUROPAEI SIVE EPICRISEOS SYSTEMATIS MYCOLOGICI EDITIO ALTERA. 755 p. Upsaliae.

(19) HALLER, A. VON
1742. ENUMERATIO METHODICA STIRPIUM HELVETIAE INDIGENARUM. 794 p., illus. Gottingae.

(20) HESLER, L. R., and WHETZEL, H. H.
1920. MANUAL OF FRUIT DISEASES. 462 p., illus. New York.

(21) HEWITT, J. L., and HAYHURST, P.
1911. DISEASES OF APPLE TREES AND FRUIT CAUSED BY FUNGI AND INSECTS. Ark. Agr. Exp. Sta. Bul. 109: 411–445.

(22) KAUFFMAN, C. H.
1918. THE AGARICACEAE OF MICHIGAN. v. 1. Lansing, Mich. (Mich. Geol. and Biol. Surv. Pub. 26.)

(23) LLOYD, C. G.
1899. CLITOCYBE MONADELPHA. Mycol. Notes 1: 17.

(24) ———
1901. CLITOCYBE TABESCENS. Mycol. Notes 1: 54.

(25) MCCARTHY, G.
1893. THE DISEASES AND INSECTS AFFECTING FRUIT TREES AND PLANTS, WITH REMEDIES FOR THEIR DESTRUCTION. N. C. Agr. Exp. Sta. Bul. 92, p. 65–144, illus.

(26) MICHELI, P. A.
1729. NOVA PLANTARUM GENERA. 234 p., illus. Florentiae.

(27) MORGAN, A. P.
1883. THE MYCOLOGIC FLORA OF THE MIAMI VALLEY. Jour. Cincinnati Soc. Nat. Hist. 6: 54–81, 97–117, 173–199, illus.

(28) MURRILL, W. A.
1911. THE AGARICACEAE OF TROPICAL NORTH AMERICA—III. Mycologia 3: 189–199.

(29) ———
1916. AGARICACEAE. No. Amer. Flora 9: 163–421.

(30) PATOUILLARD, N.
1909. ADDITIONS AU CATALOGUE DES CHAMPIGNONS DE LA TUNISIE. Compt. Rend. Cong. Soc. Savantes (Sect. Sci.) 1908: 242–257.

(31) PECK, C. H.
1888. REPORT OF THE BOTANIST. Ann. Rpt. N. Y. State Mus. Nat. Hist. (1887) 41: 51–122.

(32) ———
1892. REPORT OF THE STATE BOTANIST. Ann. Rpt. N. Y. State Mus. Nat. Hist. (1890) 44: 177–187, illus.

(33) ———
1893. REPORT OF THE STATE BOTANIST. Ann. Rpt. N. Y. State Mus. Nat. Hist. (1892) 46: 83–149.

(34) ———
1895. REPORT OF THE STATE BOTANIST. Ann. Rpt. N. Y. State Mus. Nat. Hist. (1894) 48 (1): 101–337.

(35) ———
1899. REPORT OF THE STATE BOTANIST. Ann. Rpt. N. Y. State Mus. Nat. Hist. (1897) 51 (1): 267–317, illus.

(36) ———
1900. REPORT OF THE STATE BOTANIST ON EDIBLE FUNGI OF NEW YORK, 1895–99. Mem. N. Y. State Mus. 3 (4): 140–141, illus.

(37) PERSOON, C. H.
1828. MYCOLOGIA EUROPAEA. v. 3, illus. Erlangae.

(38) PIERCE, N. B.
1892. THE CALIFORNIA VINE DISEASE; A PRELIMINARY REPORT OF INVESTIGATIONS. U. S. Dept. Agr., Div. Veg. Path. Bul. 2, 222 p., illus.

(39) PIPER, C. V., and FLETCHER, S. W.
1903. ROOT DISEASES OF FRUIT AND OTHER TREES CAUSED BY TOADSTOOLS. Wash. Agr. Exp. Sta. Bul. 59, 14 p.

(40) POLLINI, C.
1824. FLORA VERONENSIS. v. 3, illus. Veronae.

(41) RICHARDS, C. A.
1923. CULTURAL STUDIES OF ARMILLARIA MELLEA FR., AND CLITOCYBE MONODELPHA MORG. (Abstract) Science 57: 156.

(42) SACCARDO, P. A.
1901. SYLLOGE FUNGORUM. v. 15. Patavii.

(43) ———
1910. SYLLOGE FUNGORUM. v. 19. Patavii.

(44) SCHULZER VON MÜGGENBURG, S.
1883. MYKOLOGISCHES. EINIGE, MEIN ERSTES GROSSES AN DIE UNGARISCHE AKADEMIE DER WISSENSCHAFTEN ABGETRETENES BILDERWERK BETREFFENDE BERICHTIGUNGEN. Oesterr. Bot. Ztschr. 33: 255–257.

(45) SCOPOLI, G. A.
1772. FLORA CARNIOLICA; EXHIBENS PLANTAS CARNIOLIAE INDIGENAS ET DISTRIBUTAS IN CLASSES, GENERA, SPECIES, VARIETATES ORDINE LINNAEANO. Ed. 2, t. 2, illus. [Vindobonae]

(46) SCRIBNER, F. L.
1888. OBSERVATIONS THE PAST SEASON WITH GRAPE ROT AND MILDEW. Proc. N. J. Hort. Soc. 1887: 133–146.

(47) ———
1890. ROOT-ROT OF THE VINE. Orchard and Garden 12: 12–13, illus.

(48) ———
1890. FUNGUS DISEASES OF THE GRAPE AND OTHER PLANTS AND THEIR TREATMENT. 134 p., illus. Little Silver, N. J.

(49) SEYNES, J. DE
1863. ESSAI D'UNE FLORE MYCOLOGIQUE DE LA RÉGION DE MONTPELLIER ET DU GARD. 156 p., illus. Paris.

(50) STARNES, H. N.
1895. GRAPE CULTURE. Ga. Agr. Exp. Sta. Bul. 28: 229–294, illus.

(51) STEVENS, F. L., and HALL, J. G.
1910. DISEASES OF ECONOMIC PLANTS. 513 p., illus. New York.

(52) SZIGETHI-GYULA, A.
1908. TRAITEMENT DU POURRIDIÉ. Bul. Inst. Cent. Ampélol. Roy. Hongrois (1906) 1: 16.

(53) TOTTEN, H. R.
1917. ARMILLARIA MELLEA, CLITOCYBE CESPITOSA, PLEUROTUS SAPIDUS, AND CLAUDOPUS NIDULANS IN PURE CULTURE. (Abstract) Jour. Elisha Mitchell Sci. Soc. 33: 96.

(54) UNDERWOOD, L. M., and EARLE, F. S.
1896. TREATMENT OF SOME FUNGOUS DISEASES. Ala. Agr. Exp. Sta. Bul. 69: 245–272.

(55) VAILLANT, S.
1727. BOTANICON PARISIENSE. 205 p., illus. Leide and Amsterdam.

(56) VERGE, G.
1912. LE POURRIDIÉ. Prog. Agr. et Vit. 57:132–136, illus.

(57) WALKER, E.
1902. WHY APPLE TREES FAIL. Ark. Agr. Exp. Sta. Bul. 71, 32 p., illus.

(58) WILCOX, E. M.
1901. A RHIZOMORPHIC ROOT-ROT OF FRUIT TREES. Okla. Agr. Exp. Sta. Bul. 49, 32 p., illus.

(59) WINTER, G.
1884. DIE PILZE DEUTSCHLANDS, OESTERREICHS UND DER SCHWEIZ. ABT. 1. SCHIZOMYCETEN, SACCHAROMYCETEN UND BASIDIOMYCETEN. (Rabenhorst, L. Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. Aufl. 2, Bd. 1, Abt. 1. Leipzig.)

# CONTROL OF MYCELIAL NECK ROT OF ONION BY ARTIFICIAL CURING[1]

By J. C. WALKER

*Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

## INTRODUCTION

Neck rot of onion, though widespread in its occurrence and at times very destructive, is very sporadic in its appearance. It has long been attributed to one or another species of Botrytis, but not until the work of Munn[3] upon *Botrytis allii* was a distinct species unquestionably established as a cause. Further study by the writer has shown that two other species of the genus are capable of invading the neck tissues of the onion and causing symptoms sufficiently similar to those caused by *B. allii* to create confusion with the latter. Since the comparative morphology of the three species and the symptomatology of the respective diseases are to be described in a separate paper, those phases will not be taken up in detail here. For convenience the three types of decay are designated as gray mold neck rot (*Botrytis allii* Munn), mycelial neck rot (*Botrytis sp. 110*), and small sclerotial neck rot (*Botrytis sp. 108a*). During the past nine years the mycelial neck rot has been by far the most destructive in the Racine, Wis., and Chicago, Ill., onion-growing sections. *Botrytis allii* sometimes occurs, but is not generally common; *Botrytis sp. 108a* is sometimes common on white varieties, but the nature of its attack does not make it as important as *Botrytis sp. 110*. Since the control experiments to be reported herewith were conducted in the Racine district, the results pertain entirely to the mycelial neck rot.

The severity of mycelial neck rot varies widely from year to year. Infection occurs presumably about harvest time. It has been supposed that the common practice of cutting the tops at this time exposes succulent wounds to the wind-borne Botrytis spores and thus favors infection. Controlled experiments to be described later show, however, that under certain conditions as much infection may occur with the white varieties in "untopped" as in "topped" bulbs. There is little doubt that climatic variation from year to year is a very potent factor in determining the amount of infection which occurs. Moist weather at the proper time to promote sporulation of the causal organism on dead refuse and to facilitate infection of the bulbs at the harvest period is apparently necessary to cause an epiphytotic. The signs of the disease appear one to three weeks later, first, as softening of the neck tissue, followed by the appearance of gray mycelial felt upon the decayed parts. From this time the fungus advances quite rapidly, causing collapse of the tissue, and in addition to superficial closely wefted gray mycelium there develop numerous black kernel-like sclerotia, while under sufficiently high humidity a gray to dark-brown layer of conidia is produced.

It has long been generally known among onion growers that white varieties of onion are more subject to neck rot than are the red or yellow types. Munn[3] has pointed out this difference in susceptibility to *Botrytis allii*. It is equally true for the mycelial neck rot and the small sclerotial neck rot. A discussion of the nature of resistance to onion neck rot has been given in a previous paper.[4] The serious losses are therefore usually in the white varieties, and it is with these that the problem of control becomes acute. In the two sections mentioned, the White Globe is the most common white variety grown for large bulbs, while the White Portugal is grown extensively for "bottom sets."

[1] Received for publication May 29, 1924; issued April, 1925.

[2] Investigations conducted in cooperation with the Department of Plant Pathology, University of Wisconsin.

[3] MUNN, M. T. NECK ROT DISEASE OF ONIONS. N. Y. State Agr. Exp. Sta. Bul. 437, p. 361–455, illus. 1917.

[4] WALKER, J. C., and LINDEGREN, C. C. FURTHER STUDIES ON THE RELATION OF ONION SCALE PIGMENTATION TO DISEASE RESISTANCE. Jour. Agr. Research 29: 507–514. 1924.

## SCOPE OF THE INVESTIGATION

The experience of the average onion grower has led him to recognize that prompt and thorough drying or curing of the bulbs, and especially of the neck tissue, is the most practicable means of reducing neck-rot infection. In ordinary practice this condition is approached by taking advantage of clear, bright weather and natural air currents and by affording shelter during periods of rain or high humidity. However, it is not always possible to avoid the disease by these means. The purpose of the present investigation has been to determine the effect of artificial drying of bulbs after harvest upon infection, and the feasibility of this as a practicable remedial measure. As a corollary to the above, a study was made of relation of maturity of tops and of the removal of the same at harvest time to the amount of infection which occurs. All of the experiments reported, with one exception, were performed on onions grown in the Racine-Kenosha district of southeasten Wisconsin.

## EXPERIMENTAL RESULTS

### RELATION OF MATURITY OF BULBS TO INFECTION

Methods of handling the onion crop vary in different localities. In some the bulbs are pulled and the tops clipped when the neck tissue is still quite succulent; in others, the tops are allowed to desiccate appreciably before they are removed; while in still others the tops remain attached for some weeks or months of storage. It is generally supposed that, other conditions being equal, the immature succulents necks are the most favorable for the beginnings of neck rot. Some observations on this were made at Racine in 1917 and 1918.

In each year a portion of a field of Red Globe onions was selected in which the plants were maturing unevenly. When the tops of the more advanced plants were thoroughly dried down, all of the bulbs were pulled and topped in the usual manner. They were then separated into two lots, which were designated "mature" and "immature" according to condition of the neck tissue. This was done on September 17, 1917, and on September 16, 1918. In the latter year a portion of both mature and immature bulbs were clipped so as to leave about one-half inch of neck tissue, while in the remaining portion 2 to 3 inches of neck was left. The bulbs were placed in 1-bushel folding crates and, according to the customary practice, were then stacked in the field to cure for about three weeks. They were then removed to an onion warehouse for storage. The bulbs harvested in 1917 were finally examined on January 15, 1918; those harvested in 1918 were finally examined on March 8, 1919. The results are given in Table I.

TABLE I.—*Percentage of mycelial neck rot developing in bulbs harvested with "mature" and "immature" neck tissue; Red Globe variety, grown at Racine, Wis., 1917 and 1918.*

| Year and condition of neck tissue at harvest time | Length of neck left upon topping | Crate No. | Total number of bulbs included | Per cent of bulbs in which neck rot developed in subsequent storage |
|---|---|---|---|---|
| 1917 | *Inches* | | | |
| Immature | 1 | 1 | 300 | 7 |
| | | 2 | 300 | 6 |
| Mature | 1 | 3 | 300 | 3 |
| | | 4 | 300 | 3 |
| 1918 | | | | |
| Immature | ½ | 1 | 88 | 22 |
| | 2 to 3 | 2 | 91 | 23 |
| | | 3 | 90 | 18 |
| | | 4 | 84 | 23 |
| Mature | ½ | 5 | 163 | 11 |
| | 2 to 3 | 6 | 206 | 7 |
| | | 7 | 127 | 9 |
| | | 8 | 165 | 7 |

It is to be seen that in every case the percentage of neck rot in the immature bulbs was practically double that in the mature bulbs handled under identical conditions. The amount of neck tissue allowed to remain on the bulbs did not materially affect the extent of neck-rot infection, although the relative amounts of succulent tissue exposed is probably the important factor involved. In confirmation of this the writer has data for 1915 which show the Red Globe crop of onions in the Racine district was reduced from 20 to 50 per cent in storage by mycelial neck rot. The growing season of that year was unusually cool and moist, and as a result the onions matured some four weeks later than usual and in most cases were harvested before the neck tissue was thoroughly dried out. Likewise in 1924, an unusually rainy season, the losses in all varieties of onion in this and in the Chicago district due to mycelial neck rot were as great as those in 1915. As usual, the disease was most severe on the white varieties.

It is unforunate that the writer was unable to make parallel tests with a white variety. Later studies [5] have shown that the basic principle in resistance to neck rot among colored varieties of onion is due to a host toxin which is affiliated with the scale pigments and becomes functional as the outer scale tissue dies and permits its diffusion into drops of moisture on the exterior. The exposure of the succulent neck tissue of the immature bulbs often allows the neck-rot organism to gain entrance without contact with the toxin of the dead pigmented scale tissue, whereas, in the case of the mature neck, the parasite is more liable to come in contact with this toxin. With the white varieties the effect of this toxin does not enter in. Although the mature neck tissue is undoubtedly less favorable to infection than the immature neck tissue, the difference is not due, as it is in part in the colored varieties, to the presence or absence of the inhibitory toxin. More experimental work on this point is contemplated.

### EFFECT OF REMOVAL OF TOPS UPON INFECTION

In order to estimate the bearing of the removal of tops at harvest time upon infection with the mycelial neck-rot fungus, some artificial inoculations were first performed. Bulbs of White Portugal, Yellow Globe, and Red Globe varieties, the tops of which had fully matured, were harvested at Madison, Wis., on September 3 from soil which had not grown onions for at least six years. The tops were left intact on a portion of each variety, while from a similar amount of each variety the tops were cut close to the bulb so as to expose the succulent tissue. The bulbs were then sprayed with a spore suspension of the fungus. A number of each variety, topped and untopped, were sprayed with distilled water as controls. All were then placed in a moderately humid chamber at about 18° C. After 19 days the bulbs were examined, the percentage of neck rot recorded being given in Table II.

In the experiment a much larger percentage of infection occurred in topped than in the untopped bulbs, and there is little doubt that the removal of tops offers greater opportunity for infection. It is to be noted, however, that the fungus was not entirely excluded by leaving the tops intact, and the question naturally arises whether under more favorable environment even more infection might not have occurred in the untopped bulbs. The function of the inhibitive toxin already cited [5] in the pigmented neck tissue also enters into consideration when the amount of infection in colored and uncolored untopped bulbs is noted. It is significant in this connection that the highest percentage of infection in untopped bulbs occurred in the white variety. This difference becomes more striking in the field experiments to be discussed presently.

TABLE II.—*Development of mycelial neck rot in topped and untopped bulbs of White Portugal, Yellow Globe, and Red Globe onions 19 days after artificial inoculation*

| Variety | Method of handling tops | Inoculated | | Control | |
|---|---|---|---|---|---|
| | | Total number of bulbs | Per cent neck rot | Total number of bulbs | Per cent neck rot |
| White Globe | Topped | 21 | 90 | 20 | 0 |
| Do | Untopped | 19 | 16 | 20 | 0 |
| Yellow Globe | Topped | 10 | 80 | 9 | 11 |
| Do | Untopped | 10 | 10 | 10 | 0 |
| Red Globe | Topped | 10 | 80 | 10 | 0 |
| Do | Untopped | 10 | 0 | 10 | 0 |

During the years 1917, 1918, and 1920 some comparative field studies were made. Since the Red Globe onion is the type grown almost exclusively in the Racine section, observations during the first two years were limited to that variety. In 1920 special plantings of Yellow Globe and White Globe were made alongside Red Globe for the purpose of this study. In each season when the crop was mature several bushels were taken from a small portion of the field and the bulbs were impartially divided into two lots, those of one being placed in crates with the tops intact and those of the other with the tops clipped. The crates were stacked in the field to cure for several weeks and were then removed to storage. Final notes were taken two to three months later.

The data accumulated during three seasons is given in Table III. There

[5] WALKER, J. C., and LINDEGREN, C. C. FURTHER STUDIES ON THE RELATION OF ONION SCALE PIGMENTATION TO DISEASE RESISTANCE. Jour. Agr. Research 29: 507-514. 1924.

was always a difference in favor of the untopped lots with the colored varieties. These are in the main so resistant to neck rot, however, that the importance of the difference is slight even though consistent. A striking comparison between the resistance of colored and white varieties in 1920 is given. All lots were rained upon twice during curing, and environing conditions were thus quite favorable for the disease. In spite of this, the untopped colored varieties remained free from disease, while the topped bulbs showed slight amounts. With the White Globe variety heavy infection occurred and the difference between the topped and untopped lots was negligible. It appears, therefore, that when favorable conditions for infection prevail, this variety may be expected to succumb regardless of the method of handling tops at harvest. On the other hand, some advantage may be expected with the colored varieties in an average season if the tops are left intact during storage.

TABLE III.—*The effect of removal of onion tops at harvest time upon the occurrence of mycelial neck rot; experiments conducted at Racine, Wis., 1917–1920*

| Year and variety | Topped or untopped | Crate No. | Total number of bulbs included | Percentage of bulbs affected by neck rot at end of storage period |
|---|---|---|---|---|
| | | | | *Per cent* |
| 1917 Red Globe | Untopped | 1 | 271 | 1 |
| | | 2 | 234 | (a) |
| | | 3 | 207 | (a) |
| | | 4 | 219 | (a) |
| | Topped | 5 | 233 | (a) |
| | | 6 | 292 | 5 |
| | | 7 | 295 | 5 |
| | | 8 | 315 | 6 |
| | | 9 | 326 | 4 |
| 1918 Red Globe | Untopped | 1 | 136 | 4 |
| | | 2 | 156 | 2 |
| | Topped | 3 | 127 | 9 |
| | | 4 | 165 | 7 |
| 1920 Red Globe | Untopped | 1 | 365 | 0 |
| | Topped | 2 | 340 | (a) |
| Yellow Globe | Untopped | 3 | 297 | 0 |
| | Topped | 4 | 323 | 6 |
| White Globe | Untopped | 5 | 304 | 52 |
| | Topped | 6 | 338 | 56 |

(a) Less than 1 per cent.

## INFLUENCE OF ARTIFICIAL CURING UPON INFECTION

The evidence already presented has pointed to the importance of the condition of the neck tissue of the onion bulb when exposed to the fungus in determining the amount of neck-rot infection. These data, combined with general field observations, suggested the possibility that rapid artificial curing of bulbs immediately after harvest might accomplish two things favorable to the control of neck rot. It would desiccate the neck tissues immediately so as to make them unfavorable for infection; and it would permit earlier removal to storage and thus facilitate better protection of the crop from inclement weather following harvest.

Curing experiments were conducted in 1917 and the results have already been briefly reported.[6] Further experiments on a large scale were conducted in 1918, but conditions were so unfavorable for neck rot in that year that practically no disease developed even in the controls. Further experiments were run in 1923 with favorable results. It is therefore considered advisable to report the experiments of 1917 and 1923 in some detail.

A very simple arrangement for drying was used in the 1917 experiments. A small room equipped with a coal heater was arranged so that the temperature could be raised to 100 or 110° F. Air circulation was provided by raising a double window about 4 inches from the bottom and lowering it an equal amount from the top. The onions were placed in shallow layers in standard slatted crates used for storage of onion sets. These were so placed as to secure the maximum benefit from the circulating warmed air. It was not possible to keep a uniform temperature by this means. A continuous record of temperatures was kept, and in the tabulated data the total number of hours above 90° F. for any given period is stated. In 1923 a specially constructed chamber was used, through which warmed air was forced by means of an electric blower. By the latter process the same amount of drying was secured in much less time.

EXPERIMENTS WITH ONION SETS.—Two lots of White Portugal onion sets were treated in 1917. The first of these (Lot I) was grown upon soil

[6] WALKER, J. C. CONTROL OF NECK ROT AND ANTHRACNOSE OF ONION SETS. (Abstract) Phytopathology 8 : 70. 1918.

which in the previous year had produced onions for the first time. The sets were harvested on August 16 and placed in the standard shallow crates and the latter were stacked in the field to cure in the usual manner. During the next two weeks clear bright weather prevailed with no rain, so that conditions for natural curing were as good as ever occurs under Wisconsin conditions. On August 30, 12 crates of these onions were removed to the warehouse and they were divided into two lots of 6 crates each. No sign of neck rot was noted at this time, although later observations indicate that infection had already occurred. Five crates of one lot were placed in the drier on September 3, and one crate was reserved as control.

A second lot of White Portugal onion sets was secured from an adjoining field where onions had been grown for the first time. These were harvested on August 30 and were removed to the warehouse at once without the usual field curing. There was no evidence of neck rot at this time. One crate was given no further treatment, but was so placed as to afford free air circulation and protection from rain. One crate was placed in the drier on September 3, and kept there for seven days. The remaining two crates were kept damp for one week. One of these was given no further treatment; the other was placed in the drier for seven days.

After treatment, the onions were stored in a standard onion warehouse

TABLE IV.—*The effect of artificial drying upon the development of mycelial neck rot in White Portugal onion sets, 1917*

| Lot No. | History previous to experiment | Nature of treatment | Crate No. | Number of days in drier | Hours above 90° F. | Condition at end of storage period | |
|---|---|---|---|---|---|---|---|
| | | | | | | Bulbs showing neck rot | Shrinkage in weight |
| | | | | | | *Per cent* | *Per cent* |
| I | Grown on soil which had produced onions previous year for first time. Harvested Aug. 16; cured in standard crates in field for two weeks of dry, clear, and thus very favorable weather. Removed to warehouse Aug. 30. | All but crate No. 1 placed in drier Sept. 3. | 1 | 0 | (1) | 9 | 18 |
| | | | 2 | 2 | 28 | 3 | 11 |
| | | | 3 | 4 | 47 | 2 | 11 |
| | | | 4 | 6 | 58 | 3 | 11 |
| | | | 5 | 8 | 88 | 1 | 10 |
| | | | 6 | 16 | 164 | 0.5 | 11 |
| | | All artificially dampened and kept damp for one week; then all but crates 7 and 8 placed in drier Sept. 10. | 7 | 0 | (1) | 50 | 55 |
| | | | 8 | 0 | (2) | 27 | 45 |
| | | | 9 | 2 | 21 | 7 | 19 |
| | | | 10 | 6 | 57 | 9 | 17 |
| | | | 11 | 9 | 92 | 9 | 29 |
| | | | 12 | 14 | 117 | 10 | 17 |
| II | Grown on soil new to onions. Harvested Aug. 30 and removed to warehouse at once. | No further treatment | 13 | 0 | (1) | 44 | 49 |
| | | In drier on Sept. 3 | 14 | 7 | 73 | 7 | 19 |
| | | Kept damp one week. In drier Sept. 10. | 15 | 7 | 65 | 10 | 26 |
| | | Kept damp one week. No further treatment. | 16 | 0 | -------- | 92 | 97 |

[1] Untreated. [2] Dried in sun.

One crate was then removed from the drier to the warehouse at two-day intervals. In this and subsequent trials the outer scales of the bulbs appeared to be thoroughly dry after exposure of two days, but the treatment was extended much longer in order to determine any possible benefit of longer exposure.

In order to increase possibilities of infection and to simulate extreme weather conditions, the remaining six crates of sets were dampened and kept damp for one week. One crate was then set aside without further treatment. Another was dried in the sun for several days according to the usual commercial method. The remaining four crates were placed in the drier and one crate removed after 2, 6, 9, and 14 days, respectively.

at Racine. On January 4 following, final notes were taken. The percentage of neck rot was estimated by examining several hundred sets in each crate. Each crate was then given the usual commercial sorting in which all excess chaff and decayed bulbs were removed. The weight of marketable sets was then determined and the shrinkage by weight for each crate was calculated. The results are summarized in Table IV.

In spite of the good weather conditions which prevailed, 9 per cent of the bulbs in Lot I and 44 per cent of the bulbs in Lot II decayed in the untreated crates. Where either lot was exposed to damp conditions for a short period the amount of infection was in each case greatly increased. In all cases the artificial drying showed bene-

ficial results. Even in the best portion of Lot I (crates Nos. 1 to 6), where only 9 per cent neck rot appeared in the control, the amount of infection was reduced two-thirds or more. In the dampened portions of Lot I (crates Nos. 7 to 12) drying by natural agencies (crate No. 8) reduced infection from 50 to 27 per cent, but artificial curing reduced the disease to the almost negligible amount of 7 to 10 per cent. Thus, by the latter treatment, damage due to the exposure to a week's damp weather was prevented.

In Lot II, where the amount of disease reached much higher proportions, the damage from neck rot was reduced to a negligible quantity by means of artificial drying. There was almost a total loss when this lot was exposed to damp conditions (crate No. 16), but immediate artificial drying again prevented such damage (crate No. 15). Representative amounts of healthy onions at the end of the storage period from crates 13, 14, and 16 are shown in Plate 2.

In comparing the amount of neck rot in crates exposed to drying for various intervals it is noteworthy that in each case (crate No. 2 and crate No. 9) practically the maximum reduction of disease was reached within two days' exposure. By forced circulation of warm dry air, the length of treatment can undoubtedly be reduced much more.

### EXPERIMENTS WITH LARGE BULBS

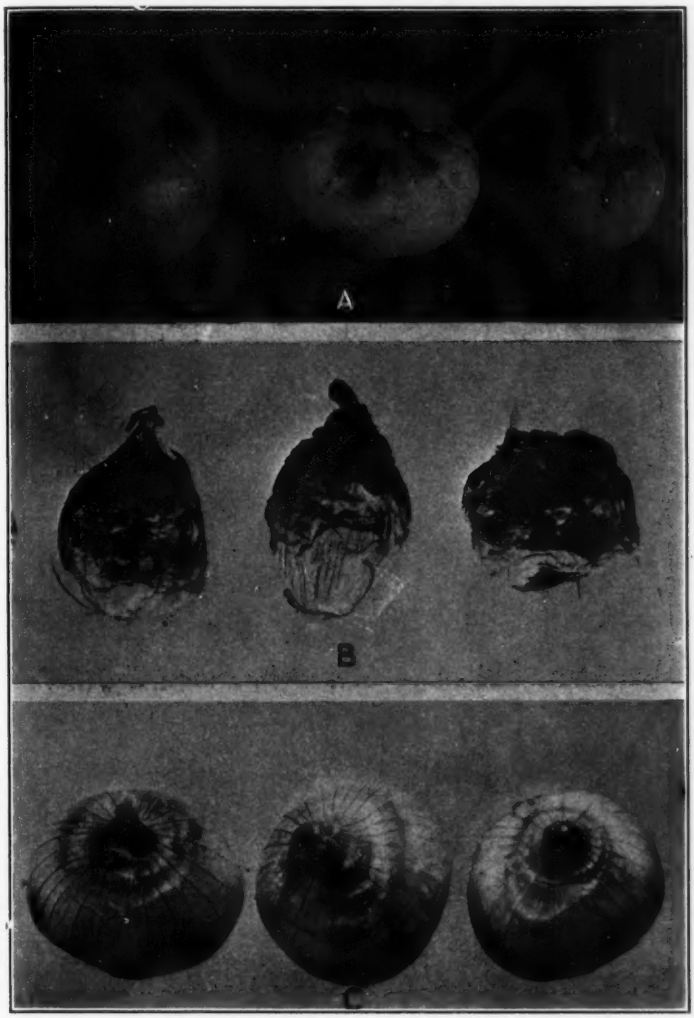
Two experiments were conducted with large bulbs, Lot III in 1917 and Lot IV in 1923. Lot III consisted of the oversize bulbs sorted out at harvest time from the sets which comprised Lots I and II. A few of these bulbs showed signs of incipient decay before they were placed in the drier. Evidence of such early infection is shown in Plate 1, A. The exposure to warm, dry air resulted in practically all cases in thorough dehydration of the diseased tissue in such bulbs. Observation throughout the storage period showed that in practically all cases this treatment checked any further advance of the fungus. The results with dried and untreated portions of Lot III are given in Table V. Almost complete elimination of loss from neck rot was attained. Typical bulbs from the dried lot in which desiccation of the slightly decayed neck occurred are illustrated as they occurred at the end of the storage period in Plate 1, B. Completely decayed bulbs, typical of the untreated lot, are shown in Plate 1, C.

Lot IV consisted of large White Globe onions grown on old onion soil at Racine, Wis., in 1923. They were harvested about September 10 and stacked in the field in slatted bushel crates for about three weeks before being placed in the drier on October 2. At the latter date there was evidence of neck rot in its early stages in a goodly percentage of the bulbs. In these the decayed tissue dried down in the same manner noted for Lot III, and in the majority of cases this resulted in a complete check of the fungus. The length of exposure and the temperature during the period of exposure with the final notes taken on December 14, after about two-months' storage are given in Table V. Although the neck tissue of large bulbs is naturally more difficult to dry out satisfactorily, it was possible to accomplish this with forced circulation of air for two or three days. The air current was maintained at room temperature, except for three to six hour intervals, during which the temperature was raised to 40° C. or 44° C.

The amount of actual decay due to neck rot was in each case reduced to an almost negligible quantity compared with that which developed in the control. The percentage of bulbs showing complete check of incipient neck-rot infection is also significant. Of course, more prompt drying of bulbs after harvest would have eliminated the incipient decay. It is valuable, however, to know that with proper treatment the disease can be checked even after the signs of the disease have become evident.

### CONCLUSIONS

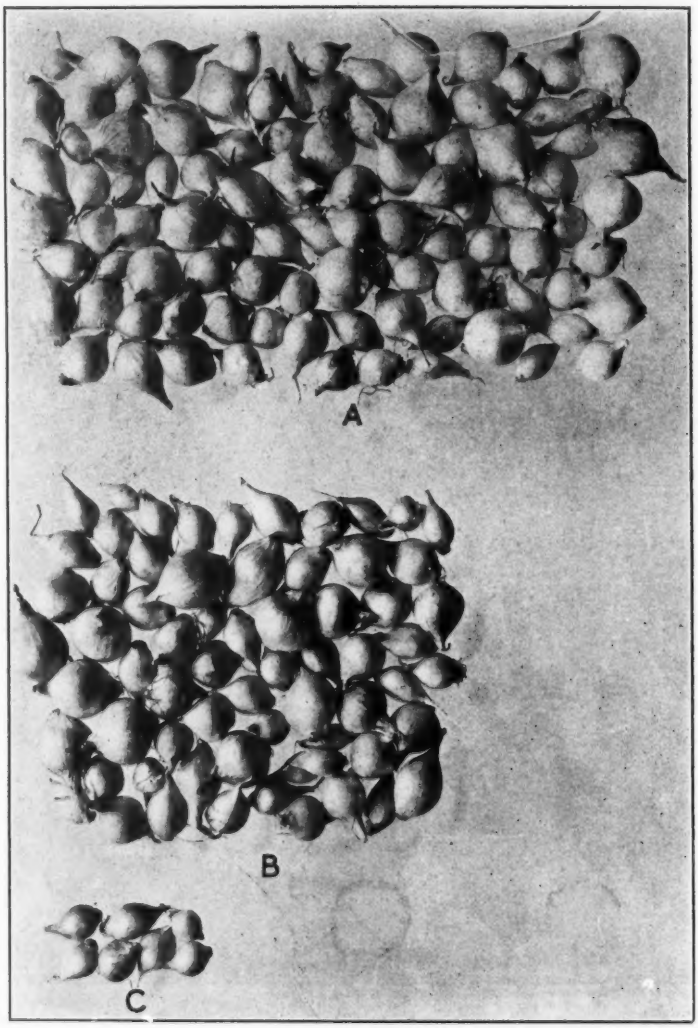
The data presented, together with field observations over a period of years, show that in the Middle West and probably in all of the Northern States several factors enter into the determination of the amount of infection by the mycelial neck rot disease. Weather conditions preceding and during harvesting and curing of crop are undoubtedly influential, and more study of their exact relation would be desirable. It is apparent that the state of maturity of the neck tissue of the bulbs at harvest is an important factor. Removal of tops may increase infection, but it is evident that this operation may under some conditions have little influence in the case of white varieties. The grower is unable by any ordinary means to so handle the crop as to prevent neck-rot infection, and this is especially the case with the white varieties, which are much more susceptible than colored varieties.




A.—Early stage in the development of the disease. Note shrinkage of the scale progressing from the neck downward, followed by the development of the gray mycelial felt on the decayed tissue

B.—Advanced stage of decay after several months in storage. Desiccation of the rotted tissue leaves dry, shriveled mummies. Note the bulb at the left is covered with conidiophores and conidia of the fungus, while the more common symptom shown in the other two bulbs consists of the large sclerotia without sporulation. Samples taken from the untreated portion of Lot III (see Table V and the text)

C.—Typical bulbs from the treated portion of Lot III. The disease was apparent at the time of drying, but the treatment resulted in desiccation of the decayed tissue at the neck and in the check of the fungus. These bulbs were photographed at the end of the storage period




The effect of artificial curing on the development of mycelial neck rot. Representative amounts of healthy onions remaining at the end of the storage period from equivalent portions of white sets taken from a common source and handled as follows:

A.—Harvested August 30, 1917, placed in drying room for 7 days beginning September 3; 7 per cent neck rot developed. (See crate 14, Table IV)

B.—Harvested August 30, protected from rain and allowed to cure naturally without any artificial treatment; 44 per cent neck rot developed. (See crate 13, Table IV)

C.—Harvested August 30, exposed to damp conditions for one week then allowed to cure without any artificial treatment; 92 per cent neck rot developed. (See crate 16, Table IV)

TABLE V.—*The effect of artificial drying upon the development of mycelial neck rot in large bulbs of White Portugal and White Globe varieties, 1917 and 1923*

| Lot No. and year | History of bulbs previous to experiment | Subsequent treatment | Crate No. | Length of drying period | Total number of bulbs | Condition at end of storage period | |
|---|---|---|---|---|---|---|---|
| | | | | | | Bulbs decayed with neck rot | Bulbs showing incipient neck rot completely checked |
| | | | | | | *Per cent* | *Per cent* |
| III 1917. | White Portugal "overruns" from Lots I and II (see Table IV), 1¼ to 1¾ inches in diameter. | Untreated | 17 | | 304 | 36 | |
| | | In drier 7 days (81 hours above 90° F.) | 18 | | 301 | .6 | |
| IV 1923. | White Globe large onions, grown on old onion soil; harvested about Sept. 10, cured in bushel crates about three weeks, then placed in drier Oct. 2. | Untreated | 19 | | 437 | 44 | 0 |
| | | In drier (with forced circulation of air). | 20 | 2½ days at room temperature and 6 hours at 40° C. | 1,093 | 9 | 19 |
| | | | 21 | 3 days at room temperature, 3 hours at 44° C. | 630 | 5 | 37 |
| | | | 22 | do | 515 | 7 | 21 |

The experimental evidence shows that mycelial neck rot may be largely prevented by thorough desiccation of the neck tissue of the bulbs within the first two to three weeks after harvest. It is shown that this may be accomplished by exposing the bulbs to higher temperature, by exposing them to forced air currents, or by a combination of the two methods. The principle of this measure of control seems to be sound. There remains to be worked out details whereby artificial curing can be applied on a large commercial scale with satisfactory rapidity and at a reasonable cost.

It is desirable that this investigation be extended to include the gray-mold neck rot *Botrytis allii*. Although this disease is ordinarily of minor consequence in the Illinois and Wisconsin sections, it is nevertheless widespread in its distribution and often causes serious losses in other sections. The life history of the causal organism is so similar to that of mycelial neck rot that the same methods of handling would probably lead to effective control.

## SUMMARY

(1) The mycelial neck rot of onion, caused by an undescribed species of Botrytis, herein referred to as *Botrytis sp. 110*, is a common cause of heavy storage losses of onion, especially of the white varieties. This paper is concerned with the relation of certain methods of handling the onion crop at harvest and in storage to infection and to the control of the disease.

(2) When removal of onion tops at harvest is practiced, the state of maturity of the plant has a direct bearing upon the amount of infection. In the experiments with the Red Globe variety the percentage of bulbs infected was doubled in the case of lots in which the neck tissue was still succulent at time of harvest as compared with those in which the tops had matured properly.

(3) In the case of colored varieties the removal of tops as compared with allowing them to remain intact after harvest usually resulted in greater infection. In the one experiment with White Globe variety there was little difference to be noted.

(4) Artificial curing of onion bulbs sufficient to cause desiccation of the neck tissue within two or three weeks after harvest has resulted in all cases in a material reduction in the amount of disease. This treatment promises to become an effective control measure, provided it can be adapted to commercial use at a reasonable cost.

# RELATIVE SUSCEPTIBILITY OF SELECTIONS FROM A FULGHUM-SWEDISH SELECT CROSS TO THE SMUTS OF OATS [1]

By GEORGE M. REED,[2] *formerly Pathologist in charge of Smut Investigations*, and T. R. STANTON, *Agronomist in charge of Oat Investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

Little is known concerning the reaction to the smuts of oats shown by selections from crosses between very resistant and very susceptible varieties. Definite information on the nature of infection, as well as on the mode of inheritance of smut resistance or smut susceptibility in such crosses, is essential for the breeding and development of smut immune varieties. The writers are cognizant of the fact that the data presented herein were obtained from studies of one cross only, but they seem conclusive enough to warrant publication. They are certainly an indication of the possibilities of the development, through hybridization, of strains of oats resistant to smut. It is evident that the production of economically desirable varieties of oats immune from, or highly resistant to, smut will be an important phase of varietal improvement in the future.

## REVIEW OF LITERATURE

The studies of Biffen (*4*) [3] clearly demonstrated that resistance of wheat to *Puccinia glumarum* is inherited in typical Mendelian fashion. Biffen crossed the susceptible Red King with the resistant Rivet. The $F_1$ generation was susceptible. The $F_2$ progeny segregated into *susceptible* and *resistant* in an approximately three to one ratio. These results of Biffen recently have been confirmed by Armstrong (*2*).

Investigations of crosses between varieties of wheat susceptible or resistant to *Puccinia graminis* have been made by a number of workers. Hayes, Parker, and Kurtzweil (*11*) found that resistance was dominant in crosses between certain varieties of *Triticum vulgare* and *T. dicoccum*. On the other hand, susceptibility was dominant in crosses of *T. vulgare* and *T. durum*. There also was a strong linkage between rust resistance and the durum characters in the $F_2$ generation. Puttick (*13*), Melchers and Parker (*12*), Aamodt (*1*), Garber (*8*), Harrington and Aamodt (*9*), and Hayes and Aamodt (*10*) have contributed additional data on the general problem of the inheritance of resistance to *Puccinia graminis*, the organism causing stem rust.

Gaines (*6*) has studied the inheritance of resistance to bunt or stinking smut (*Tilletia tritici*) of wheat. He crossed the resistant Turkey and the susceptible Hybrid No. 128, and also Turkey and Florence, both of which were resistant. In the first case, all degrees of resistance in the $F_2$ progeny were obtained. In the second cross, certain $F_3$ progenies proved to be more susceptible than either parent.

Gaines, (*7*) in a second paper on the inheritance of resistance to bunt, states that this character in wheat is not a simple Mendelian unit character but, if Mendelian, is composed of multiple factors. He further states that different wheat varieties possess different kinds of resistance, and that linkage between resistance and morphological characters is not sufficient to hinder the selection of resistant strains of any type morphologically desirable.

Wakabayashi (*18*) has studied the behavior of a cross between Red Rustproof and Black Tartar oats in reference to covered smut, *Ustilago levis*. The former variety is highly resistant while the latter is moderately susceptible. The $F_1$ and $F_2$ plants produced no smut. In the $F_3$ generation, however, 12 families out of a total of 95 were observed to contain a number of infected individuals. In no case, however, was the number very large.

[1] Received for publication June 2, 1924; issued April, 1925, as Brooklyn Botanic Garden Contribution No. 39. Data for 1919 and 1920 obtained from investigations conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Agricultural Experiment Station of the University of Idaho. Data for 1922 obtained from the Brooklyn Botanic Garden.

[2] Now curator of plant pathology at the Brooklyn Botanic Garden, Brooklyn, N. Y.

[3] Reference is made by number (italic) to "Literature cited," p. 391.

Barney (*3*) recently has reported on some studies of the inheritance of resistance to loose smut of oats in crosses between Fulghum and Black Mesdag, Swedish Select and Burt, and Turkish Rustproof and Golden Rain. The first cross involved two very resistant varieties, the second a resistant and a susceptible variety, and the third two moderately susceptible varieties. Barney suggests that resistance in the first cross depended upon three different factors, in the second upon two, and in the third upon only one. He also has shown that, in the $F_2$ generation, highly resistant as well as highly susceptible families which breed true can be isolated.

The data here presented were obtained from a cross between Fulghum and Swedish Select. Reed (*14*) and Reed, Griffiths, and Briggs (*15*), who have made extensive studies on the resistance of oat varieties to loose smut, *Ustilago avenae* (Pers.) Jens., and covered smut, *U. levis* (K. and S.) Magn., found that various strains of Fulghum were highly resistant, while strains of Swedish Select were moderately susceptible.

## RELATIVE SUSCEPTIBILITY

All of the tested strains of Fulghum and Swedish Select have been grown at one or more of the following stations: Columbia, Mo.; Ames, Iowa; Aberdeen, Idaho; Manhattan, Kans.; Pullman, Wash.; and Brooklyn, N. Y. All have been grown two or more years in the series inoculated with *Ustilago avenae*. The same is true in the series inoculated with *U. levis*, except that two strains were grown in only one year at one place.

### FULGHUM

Four different strains of Fulghum oats, including two of Kanota, have been grown. These strains, at every station in every year, have consistently shown a high degree of resistance to both *Ustilago avenae* and *U. levis*. The highest percentage of infection (2.4 per cent) by *U. avenae* was obtained at Aberdeen in 1920, and the highest percentage of infection (3.2 per cent) by *U. levis* occurred at Manhattan in 1920.

At all the different stations 10,803 plants were grown in the *Ustilago avenae* series. These showed 0.6 per cent of infection. From seed inoculated with *U. levis* 10,200 plants were grown. These showed 0.4 per cent of infection.

### SWEDISH SELECT

Six different strains of Swedish Select oats have been grown at the several stations in order to determine their susceptibility to *Ustilago avenae* and *U. levis*.

Results from inoculation with *Ustilago avenae* show that the highest percentage of infection (42.6 per cent) was obtained in strain No. 225 at Brooklyn, N. Y. The lowest percentage of infection (1.1 per cent) occurred at Brooklyn in 1922, in strain No. 226 (C. I. No. 134)[4] and also No. 227. All of the strains at some locality have had percentages of infection above 32.3 per cent except strain No. 229 (C. I. No. 1743). Of all the strains at the different stations 2,694 plants have been grown and 19.9 per cent were infected.

Results with *Ustilago levis* show that the highest percentage of infection (45.1 per cent) was obtained in strain No. 168 at Columbia. High infections of 39.7 per cent and 40.2 per cent, respectively, were obtained in strains Nos. 226 (C. I. No. 134) and 227. The lowest percentage of infection obtained was 3.4 per cent in strains No. 226 (C. I. No. 134) and 229 (C. I. No. 1743) at Brooklyn. In the entire series, 2,172 plants were grown, and 17.6 per cent of these were infected.

Where the data are sufficient for comparison there seems to be no obvious difference in the susceptibility of these strains to the two different smuts. Infections above 40 per cent are only occasional.

## PARENT VARIETIES

Fulghum and Swedish Select are important varieties of oats in the United States. They are representative of the two groups of oats to which practically all our cultivated varieties belong, namely, *Avena sativa* L. and *A. byzantina* C. Koch. Panicles and spikelets of these varieties are shown in Plate 1.

Fulghum is one of the leading varieties of so-called red oats grown in this country. As already noted, it has shown a high degree of resistance to smut. It is grown most extensively in the Southern States and in Kansas, where it has recently become popular under the name Kanota (*16*).

Swedish Select is one of our principal midseason white varieties, and is grown extensively throughout the northern United States. In the va-

[4] C. I. No. = Cereal Investigations accession number, here and throughout this report.

Panicles and spikelets of the parent varieties, Fulghum (at left) and Swedish Select (at right) (approximately two-thirds natural size)

rietal survey made by the Office of Cereal Investigations in cooperation with the former Bureau of Crop Estimates, it was estimated that 3,631,789 acres were devoted to this variety in 1919. In total acreage it is exceeded only by Silvermine and Red Rustproof.

FULGHUM

HISTORY.—Fulghum originated as a selection from the Red Rustproof variety in southeastern Georgia about 20 years ago, where it originally was grown entirely as a fall-sown variety. It has become of importance only as a spring-sown variety in recent years. A complete history of Fulghum has been recorded by Stanton (*17*).

DESCRIPTION.—Early growth semi-spreading to erect; plant very early. Culms midsized, strong, usually glabrous, 70 to 120 cm. tall. Sheaths deep green, glabrous; culm leaves midwide, margins ciliate. Peduncle midsized, straight, well exserted. Panicle equilateral, erect, small, midlong, narrow, ovate; rachis nodes 4 to 7; branches short, ascending, scabrous. Spikelets usually few, apex spikelets usually 3-flowered; kernels slender to midplump. Empty glumes 22 to 28 mm. long, 6 to 8 mm. wide, usually 8-veined, light green before maturity. Lower lemma reddish yellow (buff) 17 to 21 mm. long, glabrous, basal scar prominent to obscure; basal hairs usually present, few, short to long; awn present or absent, straight (non-twisted), 15 to 30 mm. long. Upper lemma 12 to 16 mm. long, rarely awned, usually attached to the lower, on separation the midlong, glabrous upper rachilla segment usually remaining attached to the lemma.

Fulghum is differentiated from the related Red Rustproof variety primarily by its more erect panicle, slightly less spreading habit in early growth, more slender and lighter-colored kernels with fewer awns and basal hairs. Many aberrant or so-called off-types occur in Fulghum. These vary from cultivated forms which retain their white, gray, or black kernels to the typical "false wild" oat which sheds its seeds immediately on ripening.

SWEDISH SELECT

HISTORY.—The Swedish Select variety is of Swedish origin, as the name implies, and was introduced from Russia by M. A. Carleton in 1899. The best information at hand indicates that it was introduced into Finland from Sweden and then into the St. Petersburg Province of Russia. It is said to have originated as a selection from the Ligowa oat.

DESCRIPTION.—Early growth erect; plant midseason. Culms midsized to large, strong, usually glabrous, 80 to 130 mm. tall. Sheaths deep green, usually glabrous; culm leaves midwide, margins usually glabrous. Peduncle midsized, straight, well exserted. Panicle equilateral, usually erect, midlong, midbroad, ovate; rachis nodes 5 to 7; branches short to midlong, usually ascending, scabrous. Spikelets few to numerous; usually about 50 per cent 3-flowered; kernels very plump. Empty glumes 18 to 24 mm. long, 6 to 8 mm. wide, 8 to 9 veined, deep green and slightly glaucous before maturity. Lower lemma white, 14 to 17 mm. long, glabrous, dorsal surface distinctly depressed above the awn; basal hairs usually absent; awn usually present, very dark at base, twisted and subgeniculate to twisted and geniculate, 15 to 35 mm. long. Upper lemma 10 to 14 mm. long, awnless; rachilla segment short, glabrous.

The outstanding characters of the Swedish Select variety are its short, plump kernels distinctly depressed dorsum. The awn is very dark at the base, and usually twisted and geniculate. There also is a high percentage of 3-kerneled spikelets. These characters usually serve to separate it clearly from the otherwise similar varieties, Silvermine and Lincoln.

FULGHUM-SWEDISH SELECT CROSS

HISTORY.—This cross was made by the junior author in the greenhouse at the Arlington Experiment Farm, Rosslyn, Va., in the spring of 1916, to which the hybrid number 1015 was assigned. The two $F_1$ generation plants, designated as 1015a1 and 1015a2, were grown under greenhouse conditions at the same farm, from which seed was available for sowing at the Aberdeen Substation, Aberdeen, Idaho, in the spring of 1918. A portion of the seed from a number of the resulting $F_2$ plants was turned over to the senior author for starting the studies reported herein. Panicles and spikelets of the two $F_1$ plants are shown in Plate 2.

Some brief notes were recorded on the appearance of the two $F_1$ plants when they were approaching maturity. Both were more vigorous than either parent and in general appearance they resembled Fulghum more than Swedish Select in most characters. The two plants averaged 145 cm. in height, as compared with 110 cm. and 140 cm., respectively, for the Fulghum and Swedish Select parents. The reddish tinge of the culms, empty glumes, etc., so characteristic of the Fulghum and

Panicles and spikelets of the two $F_1$ plants, 1015 a1 (at left) and 1015 a2 (at right) of the Fulghum-Swedish Select cross (approximately two-thirds natural size)

other red oat varieties, was very evident in the $F_1$ plants. The lemmas were reddish-yellow like those of Fulghum, but in shape they were rather plump like those of Swedish Select. One plant was practically awnless, while about 25 per cent of the spikelets on the other were awned. The awns were somewhat twisted and dark at the base like those of the Swedish Select parent, but only slightly bent. It is thus seen that in respect to the presence of awns this cross behaved similarly in the $F_1$ to the Sixty-Day × Burt cross, reported by Fraser (*5*).

SECOND GENERATION PROGENY

Altogether, 118 $F_2$ plants were grown from the seed of the two $F_1$ plants at the Aberdeen Substation, Aberdeen, Idaho, in 1918. Samples of these plants have been preserved and an attempt has been made to classify them with regard to the degree or extent of awning, disjunction of the second floret, and color of lemma, primarily to determine if any relationship exists between these characters and smut resistance. It is fully realized that the number of $F_2$ plants is entirely too small for the determination of genetic ratios. Further, an extended discussion on the inheritance of these characters is not within the province of this paper.

Of the 118 plants, 25 were classed as having few or no awns (awnless), 76 as having abundant awns (fully awned), and the remaining 17 as being intermediate or commonly awned (approximately 50 per cent of the spikelets awned). These numbers do not even indicate the ratio of 1 awnless, 2 partly awned, and 1 fully awned, obtained by Fraser (*5*) from a cross between an awnless strain of Sixty-Day and Burt, the latter an exceedingly variable variety with regard to awns and other kernel characters. Provided the mode of inheritance is the same in this cross, the small $F_2$ population may account for this wide deviation from the expected ratio. However, the preponderance of awns in the $F_2$ plants probably is best explained by the fact that Swedish Select usually is a rather fully awned variety, and that frequently in Fulghum as many as 50 per cent of the spikelets are awned. As a consequence, it could not be expected that the genetic behavior regarding awns would be the same as in a cross where one parent is awnless and the other only partly awned.

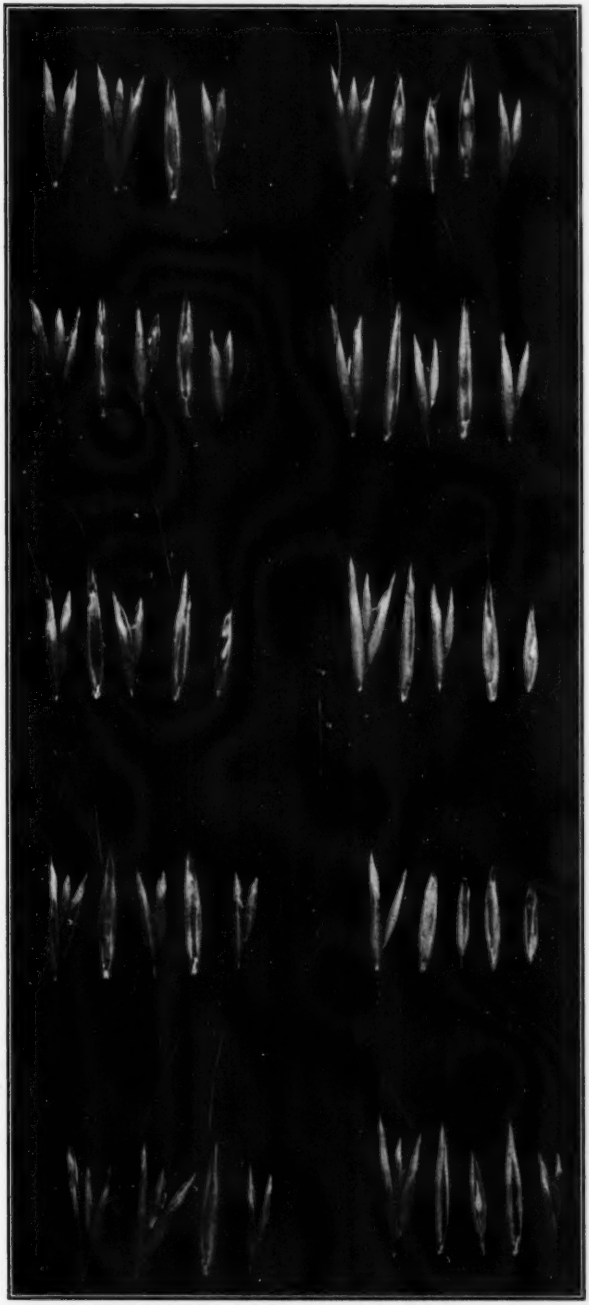
Of the 76 plants with abundant awns, about 16 showed the twisted and geniculate awn frequently occurring in the Swedish Select parent, which type of awn apparently occurs less frequently than the nontwisted straight awn of the Fulghum parent.

Classification with regard to the disjunction of the second floret or lemma of the spikelet (separation of second floret from the first) was most difficult. Those plants in which the second floret was attached rather firmly to the first, and on separation usually carried its rachilla segment with it, were classed as attached (sterilis of Fraser), while those in which the second floret disarticulated easily, the rachilla segment usually remaining on the first floret, were classed as disarticulated (sativa of Fraser). The remaining plants in which no differentiation was possible were classed as intermediate. On this basis, therefore, 25 were classed as attached, or as having the disjunction of the Fulghum, and 13 as disarticulated, or as having the disarticulation of Swedish Select, and 80 as intermediate between the two. Fraser (*5*), in his observations on the cross Sixty-Day × Burt, obtained a ratio of 1 sterilis, 2 intermediate, and 1 sativa. The numbers obtained in the present cross indicate this ratio, but do not approach it very closely.

On the basis of color of lemma, classification was even more difficult than in the case of awning and disjunction. Because of the blending of one color into the other in the same plant no very satisfactory classification could be made. The color of the lemmas varied from reddish-yellow to yellowish-white. There were really three colors present which have been described as reddish-yellow, yellow, and yellowish-white. However, differentiation between reddish-yellow and yellow, as well as between yellow and yellowish-white, was most difficult in some plants. Of the 118 plants about 32 were classed as not definitely falling into the reddish-yellow or yellow class. Of the remaining 86, there were 28 of a reddish-yellow typical of the color in Fulghum, and 58 lighter in color, and in most cases a true yellow. The dominance of the reddish-yellow color of the Fulghum over the white of Swedish Select in the $F_2$ generation of the cross in question, is indicated.

While this classification on the basis of certain kernel characters is not all that could be desired, it is believed worth while, however, in that it will indicate in some measure whether there is any relationship between morphological characters and smut resistance or smut susceptibility (pl. 3).

The spikelet descriptions of the 92 $F_2$ plants used in the smut-inoculation experiments reported herein are shown



Spikelets and florets of $F_2$ plants of the Fulghum-Swedish Select cross resistant and susceptible to the smuts of oats. Reading from left to right, 1015a1-12; 1015a2-9; 1015a1-33; 1015a2-31; 1015a1-45; 1015a2-37, resistant; 1015a1-52; 1015a2-38; 1015a1-54, susceptible, and 1015a2-54, resistant

TABLE I.—*Reaction of $F_2$, $F_4$, and some $F_3$ progenies from the hybrid No. 1015a, Avena byzantina variety Fulghum × A. sativa variety Swedish Select, to Ustilago avenae (Pers.) Jens., and U. levis (K. & S.) Magn., with descriptions of certain spikelet characters of the $F_2$ parent plants* [a]

| Hybrid number | Generation | Station and year | Spikelet characters in $F_2$ plants | | | Plants from seed inoculated with— | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Awns | Rachilla segment of second floret | Lemma color | Ustilago avenae | | | Ustilago levis | | |
| | | | | | | Total | Infected | | Total | Infected | |
| | | | | | | *Number* | *Number* | *Per cent* | *Number* | *Number* | *Per cent* |
| 1015a1–1 | $F_2$ | Aberdeen, 1919 | Abundant | Intermediate | Reddish-yellow | 35 | 4 | 11.4 | | | |
| 1a | $F_4$ | Brooklyn, 1922 | | | | 40 | 4 | 10.0 | 48 | 1 | 2.1 |
| 2 | $F_2$ | Aberdeen, 1919 | Abundant | Attached | Yellow | 74 | 18 | 24.3 | | | |
| 2a | $F_4$ | Brooklyn, 1922 | | | | 109 | 2 | 1.8 | 109 | 10 | 9.2 |
| 3 | $F_2$ | Aberdeen, 1919 | Common | Intermediate | Yellow | 54 | 8 | 14.8 | | | |
| 3a | $F_4$ | Brooklyn, 1922 | | | | 101 | 0 | 0 | 85 | 2 | 2.4 |
| 4 | $F_2$ | Aberdeen, 1919 | Common | Intermediate | Reddish-yellow | 63 | 6 | 9.5 | | | |
| 4a | $F_4$ | Brooklyn, 1922 | | | | 61 | 3 | 4.9 | 76 | 3 | 4.0 |
| 5 | $F_2$ | Aberdeen, 1919 | Few | Disarticulated | Yellowish-white | 83 | 6 | 7.2 | | | |
| 5a | $F_4$ | Brooklyn, 1922 | | | | 98 | 2 | 2.0 | 66 | 3 | 4.5 |
| 6 | $F_2$ | Aberdeen, 1919 | Abundant [b] | Attached | Yellow | 65 | 22 | 33.8 | | | |
| 6a | $F_4$ | Brooklyn, 1922 | | | | 71 | 5 | 7.0 | 115 | 0 | 0 |
| 7 | $F_2$ | Aberdeen, 1919 | Few | Intermediate | Yellowish-white | 4 | 0 | 0 | | | |
| 7a | $F_4$ | Brooklyn, 1922 | | | | 57 | 0 | 0 | 59 | 2 | 3.4 |
| 8 | $F_2$ | Aberdeen, 1919 | Common | Intermediate | Yellowish-white | 10 | 3 | 30.0 | | | |
| 8a | $F_4$ | Brooklyn, 1922 | | | | 67 | 1 | 1.5 | 34 | 3 | 8.8 |
| 10 | $F_2$ | Aberdeen, 1919 | Common | Intermediate | Yellow | 41 | 6 | 14.6 | | | |
| 10a | $F_4$ | Brooklyn, 1922 | | | | 96 | 0 | 0 | 75 | 1 | 1.3 |
| 11 | $F_2$ | Aberdeen, 1919 | Common | Intermediate | Yellowish-white | 48 | 14 | 29.2 | | | |
| 11a | $F_4$ | Brooklyn, 1922 | | | | 126 | 2 | 1.6 | 94 | 34 | 36.2 |
| 12 | $F_2$ | Aberdeen, 1919 | Common | Attached | Yellowish-white | 54 | 0 | 0 | | | |
| 12a | $F_4$ | Brooklyn, 1922 | | | | 106 | 0 | 0 | 154 | 0 | 0 |
| 13 | $F_2$ | Aberdeen, 1919 | Abundant | Intermediate | Yellowish-white | 22 | 9 | 40.9 | | | |
| 13a | $F_4$ | Brooklyn, 1922 | | | | 44 | 0 | 0 | 39 | 1 | 2.6 |
| 14 | $F_2$ | Aberdeen, 1919 | Abundant [b] | Intermediate | Yellowish-white | 70 | 0 | 0 | | | |
| 15 | $F_2$ | do | Abundant | Attached | Reddish-yellow | 62 | 10 | 16.1 | | | |
| 15a | $F_4$ | Brooklyn, 1922 | | | | 108 | 2 | 1.9 | 96 | 10 | 10.4 |
| 16 | $F_2$ | Aberdeen, 1919 | Common | Attached | Yellow | 94 | 12 | 12.8 | | | |
| 16a | $F_4$ | Brooklyn, 1922 | | | | 97 | 0 | 0 | 84 | 6 | 7.1 |
| 17 | $F_2$ | Aberdeen, 1919 | Abundant | Intermediate | Yellow | 48 | 4 | 8.3 | | | |
| 17a | $F_4$ | Brooklyn, 1922 | | | | 113 | 0 | 0 | 57 | 0 | 0 |
| 18 | $F_2$ | Aberdeen, 1919 | Few | Attached | Yellow | 20 | 0 | 0 | | | |
| 18a | $F_4$ | Brooklyn, 1922 | | | | 42 | 0 | 0 | 55 | 0 | 0 |
| 19 | $F_2$ | Aberdeen, 1919 | Abundant | Attached | Reddish-yellow | 73 | 16 | 21.9 | | | |
| 19a | $F_4$ | Brooklyn, 1922 | | | | 56 | 0 | 0 | 117 | 10 | 8.5 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | $F_3$ | Aberdeen, 1919 | Abundant | Attached | Reddish-yellow | 64 | 1 | 1.6 | | | | | |
| 21a | $F_4$ | Brooklyn, 1922 | | | | 70 | 0 | 0 | 65 | 1 | 1.5 | | |
| 22 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Yellowish-white | 4 | 0 | 0 | | | | | |
| 22a | $F_4$ | Brooklyn, 1922 | | | | 27 | 0 | 0 | 41 | 2 | 4.9 | | |
| 23 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Yellow | 54 | 25 | 46.3 | | | | | |
| 23a | $F_4$ | Brooklyn, 1922 | | | | 69 | 6 | 8.7 | 79 | 19 | 24.1 | | |
| 24 | $F_3$ | Aberdeen, 1919 | Common | Intermediate | Yellow | 35 | 7 | 20.0 | | | | | |
| 24a | $F_4$ | Brooklyn, 1922 | | | | 59 | 1 | 1.7 | 72 | 9 | 12.5 | | |
| 25 | $F_3$ | Aberdeen, 1919 | Few | Intermediate | Yellow | 68 | 10 | 14.7 | | | | | |
| 26 | $F_3$ | do | do | do | Yellowish-white | 77 | 4 | 5.2 | | | | | |
| 28 | $F_3$ | do | do | do | do | 68 | 10 | 14.7 | | | | | |
| 29 | $F_3$ | do | Abundant | do | Yellow | 59 | 5 | 8.5 | | | | | |
| 32 | $F_3$ | do | do | do | Yellowish-white | 49 | 6 | 12.3 | | | | | |
| 33 | $F_3$ | do | Few | do | do | 46 | 0 | 0 | | | | | |
| 33a1 | $F_4$ | Aberdeen, 1920 | | | | 55 | 0 | 0 | | | | | |
| 33a2 | $F_4$ | do | | | | 69 | 0 | 0 | | | | | |
| 33a3 | $F_4$ | do | | | | 104 | 3 | 2.9 | | | | | |
| 33a4 | $F_4$ | do | | | | 107 | 0 | 0 | | | | | |
| 33a5 | $F_4$ | do | | | | 70 | 0 | 0 | | | | | |
| 33a6 | $F_4$ | do | | | | 102 | 0 | 0 | | | | | |
| 33a1 | $F_5$ | Brooklyn, 1922 | | | | 60 | 0 | 0 | 50 | 1 | 2.0 | | |
| 33a2 | $F_5$ | do | | | | 53 | 0 | 0 | 53 | 0 | 0 | | |
| 33a3 | $F_5$ | do | | | | 126 | 1 | .8 | 71 | 0 | 0 | | |
| 33a4 | $F_5$ | do | | | | 77 | 0 | 0 | 110 | 0 | 0 | | |
| 33a5 | $F_5$ | do | | | | 79 | 0 | 0 | 98 | 0 | 0 | | |
| 33a6 | $F_5$ | do | | | | 71 | 0 | 0 | 97 | 0 | 0 | | |
| 34 | $F_3$ | Aberdeen, 1919 | Few | Intermediate | Yellowish-white | 75 | 11 | 14.7 | | | | | |
| 37 | $F_3$ | do | Abundant [b] | Attached | Yellow | 57 | 2 | 3.5 | | | | | |
| 37a | $F_4$ | Brooklyn, 1922 | | | | 73 | 0 | 0 | 79 | 2 | 2.5 | | |
| 38 | $F_3$ | Aberdeen, 1919 | Abundant [b] | Attached | Yellow | 42 | 1 | 2.4 | | | | | |
| 39 | $F_3$ | do | Abundant | do | Reddish-yellow | 38 | 2 | 5.3 | | | | | |
| 40 | $F_3$ | do | Few | Intermediate | Yellow | 51 | 3 | 5.9 | | | | | |
| 41 | $F_3$ | do | Abundant [b] | Attached | do | 67 | 3 | 4.5 | | | | | |
| 43 | $F_3$ | do | Abundant | Intermediate | do | 45 | 9 | 20.0 | | | | | |
| 44 | $F_3$ | do | Abundant [b] | do | Yellowish-white | 65 | 6 | 9.2 | | | | | |
| 45 | $F_3$ | do | Abundant | do | Yellow | 31 | 0 | 0 | | | | | |
| 45a1 | $F_4$ | Aberdeen, 1920 | | | | 84 | 1 | 1.2 | | | | | |
| 45a2 | $F_4$ | do | | | | 52 | 0 | 0 | | | | | |
| 45a3 | $F_4$ | do | | | | 68 | 0 | 0 | | | | | |
| 45a4 | $F_4$ | do | | | | 67 | 0 | 0 | | | | | |
| 45a5 | $F_4$ | do | | | | 77 | 0 | 0 | | | | | |
| 45a6 | $F_4$ | do | | | | 65 | 0 | 0 | | | | | |
| 45a1 | $F_5$ | Brooklyn, 1922 | | | | 109 | 0 | 0 | 86 | 1 | 1.2 | | |
| 45a2 | $F_5$ | do | | | | 71 | 0 | 0 | 99 | 0 | 0 | | |
| 45a3 | $F_5$ | do | | | | 88 | 0 | 0 | 96 | 0 | 0 | | |

[a] As used in Table I, in describing the occurrence of awns, the term "abundant" means that about 75 per cent or more of the lower florets were awned; the term "common" means that approximately from 25 to 75 per cent of the lower florets were awned, and the term "few" means that less than 25 per cent of the lower florets were awned. Under the column heading "Rachilla segment of second floret," the term "attached" means that in disjunction the rachilla segment of the second floret breaks at its base where it emerges from between the in-rolled edges of the lemma of the lower floret, and remains attached to the floret it bears. The term "disarticulated" means that the rachilla segment of the second floret disarticulates at its apex where it was attached to the floret and remains on the ventral surface of the lower floret. The term "intermediate" means that in disjunction the rachilla segment of the second floret is fractured somewhere near the middle, leaving from one-third to two-thirds of its length attached to the second floret which it bore.

[b] Awns long, twisted and geniculate, dark at base.

TABLE I.—*Reaction of $F_3$, $F_4$, and some $F_5$ progenies from the hybrid No. 1015a, Avena byzantina variety Fulghum × A. sativa variety Swedish Select, to Ustilago avenae (Pers.) Jens., and U. levis (K. & S.) Magn., with descriptions of certain spikelet characters of the $F_2$ parent plants*—Continued

| Hybrid number | Generation | Station and year | Spikelet characters in $F_2$ plants | | | Plants from seed inoculated with— | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Awns | Rachilla segment of second floret | Lemma color | Ustilago avenae | | | Ustilago levis | | |
| | | | | | | Total | Infected | | Total | Infected | |
| | | | | | | *Number* | *Number* | *Per cent* | *Number* | *Number* | *Per cent* |
| 1015a1-45a4 | $F_3$ | Brooklyn, 1921 | | | | 93 | 0 | 0 | 97 | 0 | 0 |
| 45a5 | $F_3$ | do | | | | 83 | 0 | 0 | 103 | 0 | 0 |
| 45a6 | $F_3$ | do | | | | 86 | 0 | 0 | 105 | 0 | 0 |
| 46 | $F_3$ | Aberdeen, 1919 | Abundant | Attached | Yellow | 65 | 2 | 3.1 | | | |
| 47 | $F_3$ | do | do | Intermediate | do | 54 | 1 | 1.9 | | | |
| 48 | $F_3$ | do | Few | Disarticulated | Yellowish-white | 39 | 8 | 20.5 | | | |
| 50 | $F_3$ | do | Abundant | Attached | Yellow | 84 | 10 | 11.9 | | | |
| 52 | $F_3$ | do | do | Intermediate | Yellowish-white | 54 | 19 | 35.2 | | | |
| 52a1 | $F_4$ | Aberdeen, 1920 | | | | 114 | 49 | 43.0 | | | |
| 52a2 | $F_4$ | do | | | | 111 | 28 | 25.2 | | | |
| 52a3 | $F_4$ | do | | | | 74 | 22 | 29.7 | | | |
| 52a4 | $F_4$ | do | | | | 115 | 54 | 47.0 | | | |
| 52a5 | $F_4$ | do | | | | 63 | 30 | 47.6 | | | |
| 52a6 | $F_4$ | do | | | | 84 | 45 | 53.6 | | | |
| 52a1 | $F_5$ | Brooklyn, 1922 | | | | 64 | 10 | 15.6 | 130 | 39 | 30.0 |
| 52a2 | $F_5$ | do | | | | 57 | 7 | 12.3 | 72 | 15 | 20.8 |
| 52a3 | $F_5$ | do | | | | 107 | 1 | .9 | 52 | 10 | 19.2 |
| 52a4 | $F_5$ | do | | | | 82 | 7 | 8.5 | 91 | 20 | 22.0 |
| 52a5 | $F_5$ | do | | | | 142 | 3 | 2.1 | 96 | 18 | 18.8 |
| 52a6 | $F_5$ | do | | | | 61 | 5 | 8.2 | 58 | 8 | 13.8 |
| 53 | $F_3$ | Aberdeen, 1919 | Abundant | Attached | Reddish-yellow | 73 | 9 | 12.3 | | | |
| 54 | $F_3$ | do | do[5] | do | Yellow | 74 | 43 | 58.1 | | | |
| 54a1 | $F_4$ | Aberdeen, 1920 | | | | 80 | 42 | 52.5 | | | |
| 54a2 | $F_4$ | do | | | | 104 | 53 | 51.0 | | | |
| 54a3 | $F_4$ | do | | | | 90 | 52 | 57.8 | | | |
| 54a4 | $F_4$ | do | | | | 92 | 42 | 45.7 | | | |
| 54a5 | $F_4$ | do | | | | 80 | 51 | 63.7 | | | |
| 54a6 | $F_4$ | do | | | | 93 | 73 | 78.5 | | | |
| 54a1 | $F_5$ | Brooklyn, 1922 | | | | 108 | 12 | 11.1 | 82 | 24 | 29.3 |
| 54a2 | $F_5$ | do | | | | 86 | 7 | 8.1 | 72 | 15 | 20.8 |
| 54a3 | $F_5$ | do | | | | 69 | 6 | 8.7 | 140 | 43 | 30.7 |
| 54a4 | $F_5$ | do | | | | 74 | 4 | 5.4 | 65 | 7 | 10.8 |
| 54a5 | $F_5$ | do | | | | 75 | 7 | 9.3 | 86 | 12 | 14.0 |
| 54a6 | $F_5$ | do | | | | 69 | 8 | 11.6 | 84 | 26 | 31.0 |
| 55 | $F_3$ | Aberdeen, 1919 | Abundant | Attached | Reddish-yellow | 45 | 2 | 4.4 | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 56 | $F_3$ | do | do | do | Yellow | 41 | 8 | 19.5 | | | |
| 57 | $F_3$ | do | Few | Intermediate | do | 38 | 10 | 26.3 | | | |
| 59 | $F_3$ | do | Abundant | do | do | 69 | 44 | 63.8 | | | |
| 60 | $F_3$ | do | do | Attached | Reddish-yellow | 55 | 21 | 38.2 | | | |
| 60a1 | $F_4$ | Aberdeen, 1920 | | | | 145 | 63 | 43.4 | | | |
| 60a2 | $F_4$ | do | | | | 121 | 50 | 41.3 | | | |
| 60a3 | $F_4$ | do | | | | 118 | 54 | 45.8 | | | |
| 60a4 | $F_4$ | do | | | | 91 | 37 | 40.7 | | | |
| 60a5 | $F_4$ | do | | | | 114 | 47 | 41.2 | | | |
| 60a6 | $F_4$ | do | | | | 130 | 45 | 34.6 | | | |
| 60a1 | $F_5$ | Brooklyn, 1922 | | | | 56 | 2 | 3.6 | 96 | 11 | 11.5 |
| 60a2 | $F_5$ | do | | | | 95 | 4 | 4.2 | 91 | 24 | 26.4 |
| 60a3 | $F_5$ | do | | | | 68 | 3 | 4.4 | 83 | 12 | 14.5 |
| 60a4 | $F_5$ | do | | | | 117 | 3 | 2.6 | 114 | 18 | 15.8 |
| 60a5 | $F_5$ | do | | | | 144 | 9 | 6.3 | 120 | 16 | 13.3 |
| 60a6 | $F_5$ | do | | | | 117 | 4 | 3.4 | 86 | 9 | 10.5 |
| 1015a2–1 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Yellow | 54 | 26 | 48.1 | | | |
| 2 | $F_3$ | do | Few | Attached | Reddish-yellow | 40 | 15 | 37.5 | | | |
| 3 | $F_3$ | do | do | do | Yellow | 27 | 14 | 51.9 | | | |
| 5 | $F_3$ | do | Abundant | Disarticulated | do | 72 | 19 | 26.4 | | | |
| 7 | $F_3$ | do | do | Intermediate | do | 73 | 13 | 17.8 | | | |
| 7a | $F_4$ | Brooklyn, 1922 | | | | 99 | 0 | 0 | 91 | 2 | 2.2 |
| 9 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Reddish-yellow | 45 | 0 | 0 | | | |
| 9a1 | $F_4$ | Aberdeen, 1920 | | | | 60 | 0 | 0 | | | |
| 9a2 | $F_4$ | do | | | | 63 | 2 | 3.2 | | | |
| 9a3 | $F_4$ | do | | | | 45 | 1 | 2.2 | | | |
| 9a4 | $F_4$ | do | | | | 67 | 0 | 0 | | | |
| 9a5 | $F_4$ | do | | | | 95 | 4 | 4.2 | | | |
| 9a6 | $F_4$ | do | | | | 78 | 0 | 0 | | | |
| 9a1 | $F_5$ | Brooklyn, 1922 | | | | 151 | 0 | 0 | 142 | 0 | 0 |
| 9a2 | $F_5$ | do | | | | 75 | 0 | 0 | 110 | 0 | 0 |
| 9a3 | $F_5$ | do | | | | 67 | 0 | 0 | 162 | 0 | 0 |
| 9a4 | $F_5$ | do | | | | 174 | 0 | 0 | 100 | 0 | 0 |
| 9a5 | $F_5$ | do | | | | 94 | 0 | 0 | 98 | 1 | 1.0 |
| 9a6 | $F_5$ | do | | | | 49 | 0 | 0 | 106 | 0 | 0 |
| 11 | $F_3$ | Aberdeen, 1919 | Common | Attached | Yellow | 64 | 45 | 70.3 | | | |
| 12 | $F_3$ | do | Abundant[b] | Intermediate | do | 45 | 5 | 11.1 | | | |
| 13 | $F_3$ | do | Few | do | do | 58 | 8 | 13.8 | | | |
| 16 | $F_3$ | do | Common | do | do | 73 | 9 | 12.3 | | | |
| 17 | $F_3$ | do | Abundant | Attached | do | 47 | 27 | 57.4 | | | |
| 18 | $F_3$ | do | do | Intermediate | do | 63 | 16 | 25.4 | | | |
| 18a | $F_4$ | Brooklyn, 1922 | | | | 74 | 11 | 14.9 | 59 | 8 | 13.6 |
| 19 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Yellow | 50 | 1 | 2.0 | | | |
| 20 | $F_3$ | do | do | do | do | 43 | 13 | 30.2 | | | |
| 21 | $F_3$ | do | Common | do | Reddish-yellow | 61 | 23 | 37.7 | | | |
| 22 | $F_3$ | do | Abundant | do | do | 68 | 6 | 8.8 | | | |
| 23 | $F_3$ | do | Common | do | Yellow | 49 | 2 | 4.1 | | | |
| 23a | $F_4$ | Brooklyn, 1922 | | | | 40 | 0 | 0 | 85 | 18 | 21.2 |
| 24 | $F_3$ | Aberdeen, 1919 | Common | Intermediate | Reddish-yellow | 70 | 19 | 27.1 | | | |
| 25 | $F_3$ | do | Abundant | do | Yellow | 65 | 19 | 29.2 | | | |
| 25a | $F_4$ | Brooklyn, 1922 | | | | 121 | 5 | 4.1 | 81 | 4 | 4.9 |

[b] Awns long, twisted and geniculate, dark at base.

TABLE I.—*Reaction of $F_3$, $F_4$, and some $F_5$ progenies from the hybrid No. 1015a, Avena byzantina variety Fulghum × A. sativa variety Swedish Select, to Ustilago avenae (Pers.) Jens., and U. levis (K. & S.) Magn., with descriptions of certain spikelet characters of the $F_2$ parent plants*—Continued

| Hybrid number | Generation | Station and year | Spikelet characters in $F_2$ plants | | | Plants from seed inoculated with— | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Awns | Rachilla segment of second floret | Lemma color | Ustilago avenae | | | Ustilago levis | | |
| | | | | | | Total | Infected | | Total | Infected | |
| | | | | | | *Number* | *Number* | *Per cent* | *Number* | *Number* | *Per cent* |
| 1015a2-29 | $F_3$ | Aberdeen, 1919 | Abundant | Disarticulated | Yellowish-white | 46 | 5 | 10.9 | | | |
| 30 | $F_3$ | do | do | do | do | 45 | 22 | 48.9 | | | |
| 31 | $F_3$ | do | do | Intermediate | Yellow | 49 | 0 | 0 | | | |
| 31a1 | $F_3$ | Aberdeen, 1920 | | | | 72 | 0 | 0 | | | |
| 31a2 | $F_4$ | do | | | | 90 | 2 | 2.2 | | | |
| 31a3 | $F_4$ | do | | | | 105 | 0 | 0 | | | |
| 31a4 | $F_4$ | do | | | | 65 | 3 | 4.6 | | | |
| 31a5 | $F_4$ | do | | | | 79 | 3 | 3.8 | | | |
| 31a6 | $F_4$ | do | | | | 82 | 1 | 1.2 | | | |
| 31a1 | $F_5$ | Brooklyn, 1922 | | | | 95 | 0 | 0 | 89 | 0 | 0 |
| 31a2 | $F_5$ | do | | | | 86 | 0 | 0 | 83 | 0 | 0 |
| 31a3 | $F_5$ | do | | | | 80 | 0 | 0 | 117 | 0 | 0 |
| 31a4 | $F_5$ | do | | | | 58 | 0 | 0 | 67 | 0 | 0 |
| 31a5 | $F_5$ | do | | | | 117 | 0 | 0 | 92 | 1 | 1.1 |
| 31a6 | $F_5$ | do | | | | 87 | 0 | 0 | 104 | 0 | 0 |
| 32 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Reddish-yellow | 56 | 8 | 14.3 | | | |
| 34 | $F_3$ | do | do | do | Yellow | 46 | 5 | 10.9 | | | |
| 35 | $F_3$ | do | Abundant[b] | do | Reddish-yellow | 46 | 1 | 2.2 | | | |
| 35a | $F_4$ | Brooklyn, 1922 | | | | 69 | 0 | 0 | 62 | 1 | 1.6 |
| 37 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Yellowish-white | 58 | 0 | 0 | | | |
| 37a1 | $F_4$ | Aberdeen, 1920 | | | | 110 | 0 | 0 | | | |
| 37a2 | $F_4$ | do | | | | 97 | 2 | 2.1 | | | |
| 37a3 | $F_4$ | do | | | | 81 | 1 | 1.2 | | | |
| 37a4 | $F_4$ | do | | | | 57 | 0 | 0 | | | |
| 37a5 | $F_4$ | do | | | | 87 | 7 | 8.0 | | | |
| 37a6 | $F_4$ | do | | | | 99 | 6 | 6.1 | | | |
| 37a1 | $F_5$ | Brooklyn, 1922 | | | | 133 | 0 | 0 | 94 | 0 | 0 |
| 37a2 | $F_5$ | do | | | | 110 | 0 | 0 | 99 | 0 | 0 |
| 37a3 | $F_5$ | do | | | | 90 | 0 | 0 | 104 | 0 | 0 |
| 37a4 | $F_5$ | do | | | | 87 | 0 | 0 | 133 | 0 | 0 |
| 37a5 | $F_5$ | do | | | | 72 | 0 | 0 | 84 | 0 | 0 |
| 37a6 | $F_5$ | do | | | | 121 | 0 | 0 | 141 | 0 | 0 |
| 38 | $F_3$ | Aberdeen, 1919 | Few | Intermediate | Yellowish-white | 31 | 0 | 0 | | | |
| 38a1 | $F_4$ | Aberdeen, 1920 | | | | 50 | 3 | 6.0 | | | |
| 38a2 | $F_4$ | do | | | | 56 | 0 | 0 | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 38a3 | $F_4$ | do | | | | 82 | 11 | 13.4 | | | | |
| 38a4 | $F_4$ | do | | | | 91 | 17 | 18.7 | | | | |
| 38a5 | $F_4$ | do | | | | 51 | 0 | 0 | | | | |
| 38a6 | $F_4$ | do | | | | 59 | 2 | 3.4 | | | | |
| 38a1 | $F_5$ | Brooklyn, 1922 | | | | 125 | 1 | 0.8 | 61 | 0 | 0 | |
| 38a2 | $F_5$ | do | | | | 129 | 0 | 0 | 44 | 0 | 0 | |
| 38a3 | $F_5$ | do | | | | 100 | 1 | 1.0 | 115 | 3 | 2.6 | |
| 38a4 | $F_5$ | do | | | | 74 | 0 | 0 | 78 | 5 | 6.4 | |
| 38a5 | $F_5$ | do | | | | 63 | 0 | 0 | 62 | 1 | 1.6 | |
| 38a6 | $F_5$ | do | | | | 86 | 0 | 0 | 71 | 3 | 4.2 | |
| 39 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Reddish-yellow | 54 | 23 | 42.6 | | | | |
| 40 | $F_3$ | do | do | do | Yellowish-white | 70 | 1 | 1.4 | | | | |
| 41 | $F_3$ | do | Abundant [b] | do | Yellow | 54 | 8 | 14.8 | | | | |
| 45 | $F_3$ | do | Abundant | do | do | 43 | 2 | 4.7 | | | | |
| 46 | $F_3$ | do | do | do | Yellowish-white | 85 | 6 | 7.1 | | | | |
| 46a | $F_4$ | Brooklyn, 1922 | | | | 101 | 3 | 3.0 | 51 | 4 | 7.8 | |
| 47 | $F_3$ | Aberdeen, 1919 | Few | Disarticulated | Yellowish-white | 56 | 4 | 7.1 | | | | |
| 48 | $F_3$ | do | Abundant [b] | Intermediate | Yellow | 70 | 24 | 34.3 | | | | |
| 48a | $F_4$ | Brooklyn, 1922 | | | | 64 | 0 | 0 | 64 | 5 | 7.8 | |
| 49 | $F_3$ | Aberdeen, 1919 | Abundant [b] | Intermediate | Yellow | 55 | 12 | 21.8 | | | | |
| 49a | $F_4$ | Brooklyn, 1922 | | | | 86 | 1 | 1.2 | 94 | 9 | 9.6 | |
| 50 | $F_3$ | Aberdeen, 1919 | Common | Disarticulated | Yellow | 36 | 12 | 33.3 | | | | |
| 50a | $F_4$ | Brooklyn, 1922 | | | | 86 | 3 | 3.5 | 70 | 5 | 7.1 | |
| 51 | $F_3$ | Aberdeen, 1919 | Few | Intermediate | Yellow | 60 | 3 | 5.0 | | | | |
| 52 | $F_3$ | do | Abundant | Disarticulated | Yellowish-white | 53 | 18 | 34.0 | | | | |
| 52a | $F_4$ | Brooklyn, 1922 | | | | 85 | 7 | 8.2 | 100 | 17 | 17.0 | |
| 53 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Yellow | 36 | 19 | 52.8 | | | | |
| 53a | $F_4$ | Brooklyn, 1922 | | | | 84 | 4 | 4.8 | 84 | 7 | 8.3 | |
| 54 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Yellow | 54 | 0 | 0 | | | | |
| 54a1 | $F_4$ | Aberdeen, 1920 | | | | 77 | 0 | 0 | | | | |
| 54a2 | $F_4$ | do | | | | 75 | 1 | 1.3 | | | | |
| 54a3 | $F_4$ | do | | | | 60 | 1 | 1.7 | | | | |
| 54a4 | $F_4$ | do | | | | 70 | 2 | 2.9 | | | | |
| 54a5 | $F_4$ | do | | | | 89 | 0 | 0 | | | | |
| 54a6 | $F_4$ | do | | | | 53 | 1 | 1.9 | | | | |
| 54a1 | $F_5$ | Brooklyn, 1922 | | | | 103 | 0 | 0 | 78 | 0 | 0 | |
| 54a2 | $F_5$ | do | | | | 149 | 0 | 0 | 103 | 0 | 0 | |
| 54a3 | $F_5$ | do | | | | 128 | 0 | 0 | 129 | 0 | 0 | |
| 54a4 | $F_5$ | do | | | | 59 | 0 | 0 | 79 | 0 | 0 | |
| 54a5 | $F_5$ | do | | | | 134 | 0 | 0 | 74 | 0 | 0 | |
| 54a6 | $F_5$ | do | | | | 90 | 0 | 0 | 71 | 1 | 1.4 | |
| 56 | $F_3$ | Aberdeen, 1919 | Few | Intermediate | Yellow | 21 | 7 | 33.3 | | | | |
| 57 | $F_3$ | do | Abundant | Disarticulated | Yellowish-white | 65 | 33 | 50.8 | | | | |
| 57a | $F_4$ | Brooklyn, 1922 | | | | 66 | 1 | 1.5 | 97 | 9 | 9.3 | |
| 58 | $F_3$ | Aberdeen, 1919 | Abundant | Intermediate | Yellowish-white | 55 | 14 | 25.5 | | | | |
| 58a | $F_4$ | Brooklyn, 1922 | | | | 135 | 3 | 2.2 | 75 | 18 | 24.0 | |

[b] Awns long, twisted and geniculate, dark at base.

in Table I in connection with the presentation of the data on the relative smut resistance of their progeny.

## EXPERIMENTAL DATA

The first studies on the resistance of the progeny of hybrids No. 1015a1 and No. 1015a2 to *Ustilago avenae* were conducted at the Aberdeen Substation, Aberdeen, Idaho, in 1919. The seed from 49 and 43 $F_2$ plants, respectively, was inoculated and the $F_3$ plants were examined for their behavior toward the loose smut.

In 1920, the seed from individually selected $F_3$ plants was inoculated with *Ustilago avenae* and again grown at Aberdeen. The selected plants came from rows which showed either a very high degree of resistance or of susceptibility in the previous year.

In 1922, $F_4$ plants were grown from seed harvested from the $F_3$ rows of the 1919 sowings. However, no attempt was made to grow plants from a single $F_3$ parent. In 1922, also, $F_5$ plants from the $F_4$ generation of 1920 were grown. Separate lots of seed were inoculated with *Ustilago avenae* and *U. levis*.

The methods employed have been fully described in the publications of Reed (*14*) and Reed, Griffiths, and Briggs (*15*). An adequate quantity of seed for sowing a rod row was placed in a small envelope and sufficient smut spores to thoroughly dust the seed were added.

In Table I, the data obtained on the reaction of selections from the hybrid Fulghum × Swedish Select to *Ustilago avenae* and *U. levis* are presented.

## DISCUSSION OF RESULTS

Reference to Table I shows that very great variation exists in the reaction of the different selections to the loose and covered smuts of oats. The percentage of infection ranges from zero to as high as 79 per cent. For the purpose of studying the relative resistance or susceptibility of each selection as indicated by its reaction to the smuts, some method of comparison is necessary. Accordingly, on the basis of the 1919 results, the following classes arbitrarily have been adopted and a certain range in percentage of infection assigned to each. The classes and infection ranges are as follows:

| | Per cent |
|---|---|
| 1. Very resistant | 0 to 5 |
| 2. Moderately resistant | 5 to 20 |
| 3. Moderately susceptible | 20 to 40 |
| 4. Very susceptible | 40+ |

On the basis of the data obtained for Fulghum and Swedish Select, the parents of the hybrids, the former would be placed in group 1 and the latter in group 2 or 3.

### SMUT INFECTION AT ABERDEEN, IDAHO, IN 1919

As noted in Table I, 92 $F_3$ families, the seed of which had been inoculated with *Ustilago avenae*, were grown. Of these families, 25 fall into class 1, 33 families fall into class 2, and 22 families into class 3. The remaining 12 families proved to be very susceptible and fall into class 4.

The progeny of the 2 $F_1$ plants in general has responded in a similar fashion. A few more families of hybrid No. 1015a1 are found in the very resistant class, while on the other hand, the very susceptible class is represented by an excess of families from hybrid No. 1015a2.

### SMUT INFECTION AT ABERDEEN, IDAHO, IN 1920

In 1920, the seed from six different individually selected $F_3$ plants from each of several families was inoculated and sown.

Selections were made from two resistant No. 1015a1 $F_3$ families (Nos. 33 and 45), and from five resistant No. 1015a2 $F_3$ families (Nos. 9, 31, 37, 38, and 54). All of these seven families had shown negative results in 1919. In 1920, the percentages of infection in all the selections from families Nos. 33 and 45 from No. 1015a1, and of Nos. 9, 31, and 54 from No. 1015a2, were either zero or very small. Two of the selections from family No. 37 of No. 1015a2 showed 8 per cent and 6.1 per cent infection, respectively. Two selections from family No. 38 of No. 1015a2 showed rather high infections, namely, 13.4 and 18.7 per cent. With these four exceptions, however, all of the 42 selections showed a degree of resistance comparable to the $F_3$ parent family.

Six selections also were grown from each of three susceptible $F_3$ families, Nos. 52, 54, and 60, from No. 1015a1. In 1919, family No. 52 showed 35.2 per cent infection. In 1920, the percentages of infection of the selections ranged from 25.2 per cent to 53.5 per cent. Family No. 54 in 1919 had 58.1 per cent of infection. In 1920, the selections showed infections ranging from 45.7 per cent to 78.5 per cent. Family No. 60 in 1919 had 38.2 per cent of infection and, in 1920, the selections showed infections ranging from 34.6 per cent to 45.8 per cent. These selections from very susceptible $F_3$ families showed a wider range of infection than the parents.

SMUT INFECTION AT BROOKLYN IN 1922

As noted previously, $F_4$ plants were grown at Brooklyn from seed harvested from the $F_3$ rows of the 1919 sowings, although no attempt was made to grow plants from a single $F_3$ parent. $F_5$ plants from the $F_4$ generation of 1920 also were grown. Separate sets of seed were inoculated with *Ustilago avenae* and *U. levis*. The results from *U. avenae* were much lower than might be expected. However, all the results obtained with this smut during that season were unusually low and a partial explanation is that the spores used showed much poorer germination than those employed in previous seasons. However, the results with *U. levis* in the general varietal experiments also were somewhat below those of previous years, indicating that the season was not especially favorable for these smuts.

In general, however, the $F_3$ families which had previously shown high susceptibility showed the highest infections in 1922, and the very resistant $F_3$ families showed very low infections or entirely negative results. In many cases, negative results were obtained with $F_4$ plants derived from moderately resistant $F_3$ families. The results, however, are in harmony with those previously obtained. The evidence also indicates that the selections react in a similar fashion to both smuts. Selections highly resistant to one smut also are resistant to the other, and selections highly susceptible to the one are susceptible to the other. In some cases the percentage of infection with *Ustilago levis* was too low, but the data obtained are not sufficient to prove whether or not there are cases in which the susceptibility of a selection to the two smuts is different.

RELATIONSHIP OF PLANT CHARACTERS TO SMUT RESISTANCE

The data on certain spikelet characters of the $F_2$ plants shown in Table I apparently indicate that no definite relationship or correlation exists between morphological characters and resistance or susceptibility to these smuts. (See pl. 4.)

With regard to the presence and absence of awns, it will be seen that smut resistance is not confined to those families in which the parent $F_2$ plant was either fully awned or practically awnless. Of the 12 $F_2$ plants producing immune or very resistant progeny, 7 were described as having abundant awns, 1 as being commonly awned, and 4 as having few awns. On the other hand, of the 36 $F_2$ plants producing moderately susceptible or very susceptible progenies, 24 were placed in the abundantly awned class, 7 in the commonly awned class (approximately 50 per cent of spikelets awned), and 5 in the class with few awns.

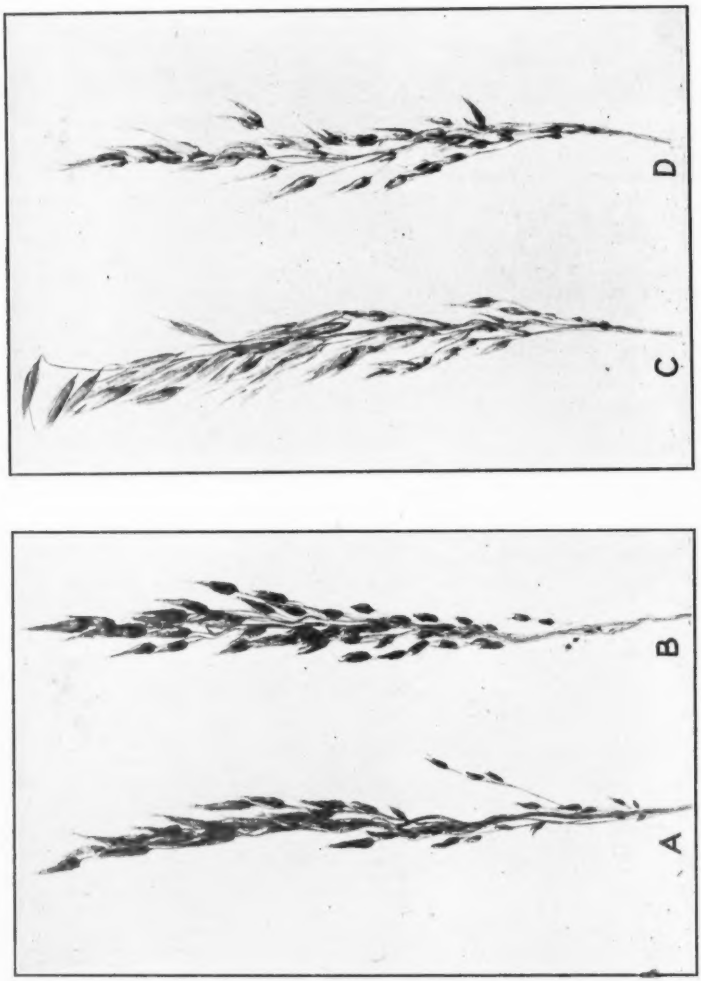
The disjunction of the second floret is discussed in terms of the disarticulation of the rachilla segment in *sativa* and its permanent attachment to the floret in *sterilis*. The 12 $F_2$ plants producing immune or very resistant progenies were classed as follows: 2 attached (Fulghum), 10 intermediate, and none disarticulated (Swedish Select). The classification of the 36 $F_2$ plants producing moderately susceptible or very susceptible progenies was as follows: 9 attached (Fulghum), 5 disarticulated (Swedish Select), and 22 intermediate.

On the basis of lemma color the 12 $F_2$ plants that produced immune or very resistant progenies, were described as follows: 1 reddish-yellow, 4 yellow, and 7 yellowish-white. The 36 plants producing moderately or very susceptible progenies were represented as follows: 6 reddish-yellow, 21 yellow, 9 yellowish-white.

It thus will be seen that no definite correlation appears to exist between morphological characters and smut resistance or smut susceptibility. Further evidence supporting this indication is shown by data recorded on the $F_3$ progenies from certain of the above very resistant and moderately susceptible or very susceptible plants which were grown in agronomic nurseries to determine their uniformity and general vigor.

For example, it was observed that, of the 14 plants grown from $F_3$ plant No. 1015a1–12,6 resembled the Fulghum parent and 8 the Swedish Select parent in most characters, thus showing the genetic segregation of morphological characters. A similar observation was made on the $F_3$ progeny from plant No. 1015a1–33. The data in Table I show that the percentage of smut in both the $F_3$ and $F_4$ progenies from the plant No. 1015a1–12 was zero. No smut was present in the $F_3$ generation of plant No. 1015a1–33, but in the $F_4$ and $F_5$, 2.9 and 0.8 per cent, respectively, of the plants of one $F_3$ selection were infected. In the $F_5$ generation 2 per cent of the plants from another $F_3$ selection of plant No. 1015a1–33 were infected with *Ustilago levis*.

Under the environment of Aberdeen, Idaho, the Fulghum variety produces rather short culms, but the Swedish Select oat grows much taller. As a re-




The smuts of oats. A and B, loose smut, *Ustilago avenae;* C and D, covered smut, *U. levis*

sult, observations were made on the relative plant height in some of the $F_3$ progenies. Of the $F_3$ progeny of $F_2$ plant No. 1015a1–45, some wereftall like Swedish Select, and others were short like Fulghum. Similar variations in plant height were indicated in some of the other families that proved very resistant to smut. In the very susceptible families No. 1015a1–52 and No. 1015a1–54, indications of marked variation in plant height also were observed.

These observations, therefore, indicate rather definitely that the plant characters of either parent are not correlated with smut resistance or smut susceptibility. The factors for smut resistance apparently are inherited independently of morphological characters in the cross in question (Fulghum × Swedish Select).

## CONCLUSIONS

1. The behavior of 92 $F_3$ families of a cross between Fulghum and Swedish Select to *Ustilago avenae* and *U. levis* has been studied. A very wide range of susceptibility of the families has been noted. A large majority of the families have shown a moderate or high degree of susceptibility Twenty-five families have shown a resistance comparable to that of the resistant Fulghum parent, and eight families a susceptibility greater than that of Swedish Select.

2. The $F_4$ selections from susceptible $F_3$ families all proved to be highly susceptible, whereas the $F_4$ selections from resistant $F_3$ families in general have been very resistant. In one case, however, two of the latter selections showed rather high percentage of infection.

3. The selections appear to behave in a similar fashion toward both loose and covered smut. These results are in harmony with those obtained by Reed (*14*) and Reed, Griffiths, and Briggs (*15*) in connection with their studies on the varietal resistance of oats to loose and covered smuts. Very few varieties of oats have been found which show any marked differences in their resistance to the loose and covered smuts.

4. There appears to be no correlation between morphological characters of the various selections and their susceptibility to the smuts. Certain $F_3$ families with the general characters of Fulghum have been found to be highly susceptible. On the other hand, some families more or less similar to Swedish Select have proved to be highly resistant. Thus it is possible to obtain the desired combination of resistance to smut and other varietal characters of oats.

## LITERATURE CITED


(1) AAMODT, O. S.
1923. THE INHERITANCE OF GROWTH HABIT AND RESISTANCE TO STEM RUST IN A CROSS BETWEEN TWO VARIETIES OF COMMON WHEAT. Jour. Agr. Research 24: 457–470, illus.

(2) ARMSTRONG, S. F.
1922. THE MENDELIAN INHERITANCE OF SUSCEPTIBILITY AND RESISTANCE TO YELLOW RUST (PUCCINIA GLUMARUM ERIKSS. AND HENN.) IN WHEAT. Jour. Agr. Sci. 12: 57–96.

(3) BARNEY, A. F.
1924. THE INHERITANCE OF SMUT RESISTANCE IN CROSSES OF CERTAIN VARIETIES OF OATS. Jour. Amer. Soc. Agron. 16: 283–291, illus.

(4) BIFFEN, R. H.
1907–1912. STUDIES IN THE INHERITANCE OF DISEASE RESISTANCE. I–II. Jour. Agr. Sci. 2: 109–128, 1907; 4: 421–429, 1912.

(5) FRASER, A. C.
1919. THE INHERITANCE OF THE WEAK AWN IN CERTAIN AVENA CROSSES AND ITS RELATION TO OTHER CHARACTERS OF THE OAT GRAIN. N. Y. Cornell Agr. Exp. Sta. Mem. 23, p. 629–676, illus.

(6) GAINES, E. F.
1920. THE INHERITANCE OF RESISTANCE TO BUNT OR STINKING SMUT OF WHEAT. Jour. Amer. Soc. Agron. 12: 124–132.

(7) ———
1923. GENETICS OF BUNT RESISTANCE IN WHEAT. Jour. Agr. Research 23: 445–480, illus.

(8) GARBER, R. J.
1922. INHERITANCE AND YIELD WITH PARTICULAR REFERENCE TO RUST RESISTANCE AND PANICLE TYPE IN OATS. Minn. Agr. Exp. Sta. Tech. Bul. 7, 62 p., illus.

(9) HARRINGTON, J. B., and AAMODT, O. S.
1923. THE MODE OF INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS WITH RELATION TO SEED COLOR IN CROSSES BETWEEN VARIETIES OF DURUM WHEAT. Jour. Agr. Research 24: 979–996, illus.

(10) HAYES, H. K., and AAMODT, O. S.
1923. A STUDY OF RUST RESISTANCE IN A CROSS BETWEEN MARQUIS AND KOTA WHEATS. Jour. Agr. Research 24: 997–1012, illus.

(11) ——— PARKER, J. H., and KURTZWEIL, C.
1920. GENETICS OF RUST RESISTANCE IN CROSSES OF VARIETIES OF TRITICUM VULGARE WITH VARIETIES OF T. DURUM AND T. DICOCCUM. Jour. Agr. Research 19: 523–542, illus.

(12) MELCHERS, L. E., and PARKER, J. H.
1922. INHERITANCE OF RESISTANCE TO BLACK STEM RUST IN CROSSES BETWEEN VARIETIES OF COMMON WHEAT (TRITICUM VULGARE). (Abstract) Phytopathology 12: 31–32.

(13) PUTTICK, G. F.
1921. THE REACTION OF THE $F_2$ GENERATION OF A CROSS BETWEEN A COMMON AND A DURUM WHEAT TO TWO BIOLOGIC FORMS OF PUCCINIA GRAMINIS. Phytopathology 11: 205–213.

(14) REED, G. M.
1920. VARIETAL RESISTANCE AND SUSCEPTIBILITY OF OATS TO POWDERY MILDEW, CROWN RUST AND SMUTS. Mo. Agr. Exp. Sta. Research Bul. 37, 41 p., illus.

(15) REED, G. M., GRIFFITHS M., and BRIGGS, F. N.
1924. VARIETAL SUSCEPTIBILITY OF OATS TO LOOSE AND COVERED SMUTS. U. S. Dept. Agr. Bul. 1275. [In press.]

(16) SALMON, S. C., and PARKER, J. H.
1921. KANOTA: AN EARLY OAT FOR KANSAS. Kans. Agr. Exp. Sta. Circ. 91, 13 p., illus.

(17) STANTON, T. R.
1921. FULGHUM OATS. U. S. Dept. Agr. Circ. 193, 11 p., illus.

(18) WAKABAYASHI, S.
1921. A STUDY OF HYBRID OATS, AVENA STERILIS × AVENA ORIENTALIS. Jour. Amer. Soc. Agron. 13: 259–266.